SERUM PROLACTIN ASSAY IN MENSTRUAL DYSFUNCTION WITH INFERTILITY

THESIS

for

MASTER OF SURGERY

(OBSTETRICS AND GYNAECOLOGY)

BUNDELKHAND UNIVERSITY
JHANSI (U. P.)

1998 ***Poonam***

C E R T I F I C A T E

This is to certify that the work entitled " SERUM PROLACTIN ASSAY IN MENSTRUAL DYSFUNCTION WITH INFERTILITY ", which is being submitted as a thesis for M.S.(Obstetrics & Gynaecology), by DR. POONAM has been carried out under my direct supervision and guidance in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi.

She has put necessary stay in the Department as required by the regulation of Bundelkhand University Jhansi.

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C O N T E N T S

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INTRODUCTION

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Classification and determination of aetiology of infertility in females have been based on various investigative procedures such as clinical evaluation, endometrial biopsies, hysterosalpingography, endoscopy and endocrine studies.

Menstrual dysfunction with infertility is a distinct clinical entity (d/t) derangement in function of the hypothalamo-pituitary ovarian-uterine axis thereby resulting in absence of menstruation for a variable period. But to locate the site of dysfunction and as well as to search for the cause of the disorder has been a matter of great concern and challenging task before the clinician within the last few years. Knowledge of the basic gynaecological endocrinology has been of paramount significance in the investigative approach in cases of menstrual dysfunction with infertility.

Thus the role of endocrinologic evaluation by RIA appears to be a very sound and plausible answer for evaluation. Recent advances in reproductive endocrinology has increminated the anterior pituitary polypeptide hormone prolactin in the pathogenesis of anovulation in amenorrhoea galactorrhoea syndrome and other menstrual dysfunctions with infertility.

Prolactin is a polypeptide hormone has 198 aminoacids. Prolactin is secreted by pituitary lactotrophs which co-exist with G.H. producing cells selectively located in the lateral wings of the pituitary gland. It is now apparent that many cases of amenorrhoea, oligomenorrhoea, corpus luteum defect and infertility are due to prolactin-secreting pituitary tumors. Lactotrophic cells are most prominent in the lateral wings and markedly increase in pregnancy or hyperestrogenic state. Extra-pituitary sites of prolactin production include decidua, endometrium, hypothalamic neurons, intestinal tract cells, lung and kidney carcinoma cells. After secretion, prolactin circulates in three molecular sizes. These heterogenous forms include :-

i) Monomeric or 'native' or 'little' prolactin (mol wt. 22,500),

ii) 'Big' prolactin (mol wt. 50,000), and

iii) 'Big-big' prolactin (mol. wt. more than 1,00,000).

Eighty percent of prolactin's immuno-reactivity separates out as the monomeric 'native' form. 'Big-big' prolactin may be secreted directly from the pituitary gland, or it may represent hormone aggregations in the circulation. In some bioassay systems, big-big prolactin appears to be biologically inactive. An elevation of an immuno-reactive form that has minimal biologic effect could explain why some patients with high prolactin levels have no galactorrhoea.

Elevated levels of the 'big' molecules are found in pregnancy, whereas patients with prolactinomas have a higher percentage of the 'little' molecules. Some women with hyperprolactinaemia and normal menstrual cycles have markedly increased levels of big-big prolactin and normal levels of monomeric prolactin. There may be inter-conversion between and aggregation of these different forms. At present, the clinician can assume that most immuno-assayable prolactin is in the native form and has significant biologic effect.

Radio-immunoassay for prolactin is a highly sensitive and precise method for the measurement for prolactin in biologic fluids. Prolactin circulates unbound in serum with a 20-minute half life and depends on the liver and kidney for its metabolic clearance. Prolactin concentrations in normal adult men and women are in the range of 4.25 ng/ml to 27.5 ng/ml.

In adulthood, concentrations are slightly but significantly higher in women than in men. A small variation in serum prolactin concentrations during the menstrual cycle, with slightly greater concentrations in the luteal phase as compared to the follicular phase, and maximal concentrations occurring at the time of LH peak, has been observed. A progressive increase in maternal serum prolactin concentration occurs during pregnancy, with mean of 30 ng/ml in the first week post-partum, the prolactin level is elevated to approximately 3 times normal, and further episodic increases

upto 100% are associated with suckling. During the second through sixth weeks post-partum the prolactin level remains approximately twice normal, with a 6 to 20 fold increase with suckling. Twelve weeks after delivery, the serum prolactin level is normal, with little or no response to suckling. In normal physiologic states, therefore, an elevated level of prolactin is required for the establishment of lactation but not for its maintenance.

Stress and breast manipulation are both potent stimulators of prolactin release. There is a sleep-associated increase in serum prolactin (upto 50%) increase with a peak between 1.00 and 5.00 a.m. Loss of this normal circadian rhythm has been described in patients with pituitary micro-adenomas. Prolactin is normally secreted in an episodic pulsatile fashion with day to day variation.

Physiological actions :

Circulating prolactin exerts its effect on target tissues via membrane receptors, followed by altered intra-cellular actions. Prolactin receptors have been localized in many tissues, including mammary glands, hypothalamus, ovary, adrenal gland, liver, kidney, testes, pancreas and lymphoid tissue.

The actions of prolactin in human female reproduction are as follows :

Menarcheal years : Mamotrophic

Ovulation : Ovarian steriodogenesis, and luteal function.

Pregnancy : - Amniotic fluid osmoregulatory function.

- Maturation of lung surfactant,

- Development of breast for lactation.

Puerperium : Lactation.

Role of prolactin in ovarian function and menstrual cycle :

Serum prolactin levels during the menstrual cycle are found to be significantly higher in the luteal phase in comparison to the pre-ovulatory phase of cycle.

In the antral follicle, in addition to the induction of LH receptor development, FSH has been shown to induce specific prolactin receptors on the granuloso cells. Prolactin in optimal concentration appears to promote aromatase action in the granulosa cells, just as optimal concentration of androstenedione and testosterone and facilitate E_2 synthesis by the granulosa cells. Prolactin is always present in the follicular fluid, although concentrations progressively decrease during folliculogenesis and are lowest in the pre-ovulatory follicle. In pituitary adenomas, intrafollicular prolactin concentrations elevated. Supraphysiologic concentration of intrafollicular prolactin concentration promotes an abnormal 5-alpha reductase action in the

granulosa, and thus interfere with FSH-induced aromatase action. Evidently, this altered enzyme milieu results in conversion of Testosterone to the irreversible androgen, namely DHT and E_2 synthesis by the granulosa is severely affected. Thus the outcome of high intrafollicular prolactin concentration is follicular atresia. These subjects are refractory to exogenous gonadotropins. Thus hyperprolactinaemia interferes with ovarian function by its inhibitory action on the Gn RH, & very severe degree of prolactin excess by increasing the intrafollicular concentration of prolactin, may exert an inhibitory influence on follicular development at the level of ovary itself. In contrast, when present in higher concentrations, prolactin may inhibit progesterone synthesis. Luteal phase defects associated with hyper-prolactinaemia probably result from a disruption of Gn RH and subsequent gonadotropin secretion, rather than from an action on the ovary.

Neuro-endocrine control of prolactin :

Pituitary prolactin secretion is under tonic hypothalamic inhibitory control. The major hypothalamic 'prolactin inhibitory factor' (PIF) appears to be dopamine. Dopaminergic neurones are present in high concentration in the medial basal hypothalamus in close contact with Gn RH neurons. Dopamine appears to act directly on the lactotrope to inhibit prolactin release.

Prolactinomas are the result of a hypothalamic defect in dopamine production or the interruption or occlusion of portal flow to a selected region or occlusion of portal flow to a selected region of the pituitary which is then revascularized from accessory vessels. If a small area of the pituitary is deprived of the portal blood supply from the hypothalamus, the resultant lack of dopamine may result in hypertrophy and hyperplasia of the lactotropes in that area of the pituitary. This process might eventually lead to adenoma formation. Dopamine appears to inhibit gonadotropin release. Gn RH secretion will reflect a balance of noradrenergic excitation and dopaminergic inhibition. Estrogens apparently promotes the release of prolactin in two fashions : (i) inhibition of dopamine at the hypothalamic level, (ii) augmentation of the release of prolactin from the pituitary by a direct action on the pituitary lactotropes. Elevated prolactin in turn exerts a positive feedback influence in the dopaminergic neurons. Thus an element of estrogen feedback effects may involve modulation of the inhibitory influence of dopaminergic neurons on Gn RH release. A similar mechanism may be involved in the well-known inverse relationship between prolactin and gonadotropin secretion. Prolactin stimulates dopamine neuronal activity in the median eminence. Hyperprolactinaemia, by elevating the dopamine levels, leads to suppression of basal LH but not FSH secretion. Hyperprolactinaemia may stimulate the release of dopamine through

a short-loop feedback mechanism. The resulting increase in dopaminergic inhibition may then alter the pattern of Gn RH secretion, leading to a reversed LH/FSH ratio, anovulation and amenorrhoea. Serotonin could decrease PIF and stimulate prolactin secretion from the pituitary or conceivably serotonin could stimulate or increase a prolactin-releasing factor.

Thyrotropin releasing hormone (TRH) cause a marked release of pituitary prolactin, secondary to a reduction in hypothalamic PIF.

Endorphins inhibit the release of dopamine into the portal blood suggest that opiates may raise prolactin levels by derising the tonic inhibition of prolactin release by the dopaminergic neurones. The prolactin releasing factors described are : TRH, serotonin, estrogen, and gama-aminobutyric acid.

Causes of Hyperprolactinaemia :

a) Physiological -

Sleep, exercise, stress, sexual excitement, luteal phase.

b) Pharmacological -

Estrogens, phenothiozine, Metaclopromide, Resorpine, Methyldopa, Cimetidine, general anesthesia.

c) Pathological -

. Neoplastic, destructive and inflammatory lesion of hypothalamus.

- Pituitary stalk compression or section.
- Prolactinoma and other pituitary tumors.
- Primary hypothyroidism, Parkinsonism.
- Chest wall injury, trauma, burns, herpes.
- PCOD, cirrhosis, chronic renal failure.
- Ectopic production of prolactin by malignant neoplasms.

Clinical manifestations of Hyperprolactinaemia :

- Amenorrhoea, oligomenorrhoea, galactorrhoea,
- Infertility, delayed puberty,
- Oligospermia, decreased libido, sexual dysfunction.

Any aetiology which leads to lesion in the sex center for the median eminence, lead to decreased secretions of all adenohypophysial incretions with the exception of prolactin which instead is inhibited by a special factor. PIF result in absence of gonadotropin releasing factors FSHRF and LHRF. This gives rise to amenorrhoea and to ovarian atrophy, whereas as a result of absent PIF activity the adenohypophysis produces prolactin.

Hence it is crucial for the early diagnosis and long term management. In an attempt to gain more insight into the possible role of prolactin excess in infertile women of Bundelkhand, this study has been undertaken.

A patient with hyperprolactinaemia presents with a broad spectrum of signs and symptoms to a variety of sub-specialities. The importance at hyperprolactinaemia in menstrual disturbances and infertility is well recognized classical presentation described with hyperprolactinaemia in secondary amenorrhoea with or without galactorrhoea. In clinical practice, it is not uncommon to see a patient with hyperprolactinaemia presenting with regular anovulatory cycles, oligomenorrhoea, primary amenorrhoea or luteal phase defect.

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AIMS AND OBJECTIVES

The determination of prolactin is used for functional check on the regulatory mechanism between the hypothalamus, the pituitary gland and gonads. Serum prolactin assay in cases of primary and secondary sterility with :

A. Menstrual pattern of :

a) Primary amenorrhoea,

b) Secondary amenorrhoea,

c) Oligomenorrhoea,

d) Irregular menstrual cycles,

e) Regular anovulatory cycles,

f) Menorrhagia.

B. Galactorrhoea.

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REVIEW OF LITERATURE

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Prolactin is unique among the anterior pituitary hormones in at least two respects : It is the only one under tonic inhibitory controls by the hypothalamus and its actions are not limited to just one or a few physiologic events (Nicoll, 1980).

Moreover the assay of prolactin in serum is undoubtedly one of the most useful assays for tumour related antigens as a prolactinoma may be present in 25 to 34% (Kleinberg et al, 1977) of women with amenorrhoea and/or galactorrhoea and hyperprolactinaemia. Hence it is crucial for the early diagnosis and long term management.

Majority of patients with hyperprolactinaemia were poorly oestrogenised supporting the assumption that prolactin exerts a negative peripheral effect on ovarian oestrogen production (Jacobs et al, 1976 and Sinha, G. et al, 1989).

Serum prolactin level is directly proportional to the size of the prolactinoma. Hence any patient with PRL level greater than 1000 u/v/ml should be thoroughly investigated (Franks and Jacobs, 1983).

After studying the pelvic anatomy, the next infertility factor to be investigated by endopelvic

sonography is the endocrine cause. When endometrial dynamic changes are confirmative to follicular maturation of luteal function, endocrine etiology of infertility remain practically ruled out (Raja, R. and Rajan, V, 1991).

The relation of serum prolactin with ovum maturation and ovulation is still unexplained and needs further elucidation. The central action of prolactin is much thought about, but as described by Bohnet et al (1976) the peripheral action like the prolactin concentration in the follicular fluid requires further attention and much analytic study.

Luteotrophic hormone or LTH was first described by Evans (1939) and its existence was subsequently confirmed by Albert and his colleagues and by Cashida and associates.

It has been well recognized that a good proportion of hyperprolactinaemic subjects will be harbouring a prolactin secreting pituitary adenoma (prolactinoma) (Gomzel and Wang, 1979; Quigley and Hand, 1980; Corunblum, 1979). The incidence of tumours in patients with amenorrhoea and hyperprolactinaemia is between 36% (Franks et al, 1977) and 48% (Kleinberg et al, 1977).

Measurement of Serum Prolactin -

Serum prolactin was measured in duplicate by a double antibody radioimmunoassay. The reagents used for

the assay were supplied by the National Pituitary Agency (VLS2 antiserum and VLS2 for radioiodination and standard) and the purified human prolactin (2.5 ug) was labelled with ^{125}I by the method of Greenwood et al (1963).

The iodinated hormone was purified using Sephadex G 50 filtration and then further using Sephadex G 50 filtration and then further purified using Sephadex G 100 filtration on the day of the assay. The second antibody was purchased from Wellcome (U.K.).

The sensitivity of the assay was 1.6 ug/ml and the intra-assay variation of a single sample measured in duplicate 20 times was 5.8 percent. The inter-assay variation of a single normal sample measured in duplicate in 15 consecutive assays was 11.2 per cent.

The range for serum prolactin found in 78 normal premenopausal women not taking oral contraceptives was 4.6 to 16.6 mg/ml (mean 10.6 ug/ml.) (Pepperell et al, 1976).

Urinary oestrogens were measured by the method of Brown et al (1968) and urinary pregnanediol was measured by the method of Cox (Cox, 1963; Barrett and Brown, 1970). Ovarian responses were assessed on the basis of these assays as follows :

1. No response : the weekly oestrogen and pregnanediol values remained below 10 ug/24 hours and 1.0 mg/24 hours respectively.

2. Follicular activity but no ovulation : the oestrogen values showed definite rises above 10 ug/24 hours but the pregnanediol values remained persistently below 1.0 mg/24 hour.

3. Follicular activity, possible ovulation, but deficient corpus luteum function : The oestrogen values showed definite rises exceeding 15 ug/24 hours and the pregnanediol values rose above 1.0 mg/24 hours but did not reach 2.0 mg/24 hours and bleeding ensured.

4. Ovulation : the oestrogen values showed definite rises and the pregnanediol value reached 2.0 mg/24 hours or more.

Patient Profile in Hyperprolactinaemia -

Prolactin is unique among the anterior pituitary hormones in at least two respects : It is the only one under tonic inhibitory controls by the hypothalamus and its actions are not limited to just one or a few physiologic events (Nicoll, 1980). As a result of this, clinical abnormalities are most likely to occur when there is loss of the inhibitory control and the consequences - while sometimes subtle - are apt to be seen in a wide range of clinical settings. Alterations in galactorrhoea, with disorders of the menstrual cycle such as amenorrhoea, oligomenorrhoea and with both male and female infertility - including some instances previously termed idiopathic.

Evidence now suggest that elevated prolactin levels may play a role in premenstrual discomfort, anxiety, PCOD and oesteoporosis.

It has been well established that hyperprolactinaemia can lead to a series of alterations in the menstrual cycle from the classic amenorrhoea to galactorrhoea syndrome. Though amenorrhoea does not always occur in hyper-prolactinaemic patients, a normal cycle or a variety of alterations like hypomenorrhoea, hypo-oligomenorrhoea, hypermenorrhoea may be present. In these cases, the negative effect of hyperprolactinaemia is represented by a selective inhibition of ovulation, luteal insufficiency or short luteal phase (De Pozo, 1976). It is therefore possible to affirm that the subjective response of female reproductive axis shows a larger degree of sensitivity to hyper-prolactinaemia. So the measurement of serum prolactin levels alone is a definitive stage in the exclusion of such a syndrome in subjects with different menstrual disorders.

Kleinberg et al (1977). Though the clinical symptoms do not always correlate with the prolactin level and patients with normal prolactin may have pituitary adenomas. The highest prolactin levels however, are associated with amenorrhoea with or without galactorrhoea. Speroff et al (1979) have observed that women with untreated hyperprolactinaemia whether or not they have an identifiable tumor are unlikely to have progression of their disease.

They may in fact have clinical and radiographic improvement. This means that they do not have to be treated with long term dopamine agonists.

Since chronic anovulation and disordered LH-FSH secretion appear to be basic features of PCOD, it is likely that endorphine liberation in the hypothalamus suppresses both dopamine and Gn-RH pathways leading to hyperprolactinaemia. High prolactin levels also inhibit oestrogen synthesis in the ovary leading to chronic anovulation and PCOD.

Increased prolactin levels produces anovulation because it prevents the LH pulsatility and interferes with the positive feedback action of estradiol at the hypothalamic level through blockage of the estrogenic receptors. This chronic anovulation leads to oligomenorrhoea and other menstrual disorders. Hypothalamic resistance to the effect of oestrogens appears to be mediated by central receptors to progesterone in conjunction with dopaminergic mechanism (Fluekiger et al, 1982). The action of prolactin on the ovary may be due to a decreased number and affinity of the LH receptors in the corpus luteum with an associated decrease in the production and secretion of progesterone. Ovarian sensitivity to prolactin is extremely variable and that moderately elevated levels may have no effect or may cause luteal insufficiency in some cases and amenorrhoea in others.

Hyperprolactinaemia in a woman with regular ovulatory menstrual cycles has been observed by Isao and Kyogo (1985).

Recently, Robert Harrison (1988) has reported stress spikes of hyperprolactinaemia in couples with unexplained infertility. Pregnancy rate in such women when treated with clomiphone citrate and bromocryptine were found to be significantly more successful than placebo. Anterior pituitary dysfunction due to hyperprolactinaemia during luteal phase following ovarian hyperstimulation has been observed by Hannu et al (1987). Though transient hyperprolactinaemia appears to be common in women attending infertility or general Gynaecologic clinics. Though a hyperprolactinaemia at rest test limited to 3 blood samples for only prolactin determination at 0, 03 and 60 minute, should suffice to diagnostic pitfalls and unnecessary treatment (Muneyyirei et al, 1989).

Visual Complications -

Women with pituitary tumours may develop visual complications during pregnancy. Mostly these develop late in pregnancy, but they may also occur during the first two trimesters. Visual-field defects have been reported in pregnant patients with pituitary tumours who had been given ovulation-inducing treatment with human gonadotrophins. These reports are mainly of individual cases and give no information on the incidence of the complication. Gemzell induced ovulation with human gonadotrophins in about 700 anovulatory women, resulting in 250 full term pregnancies. Only three of them were complicated by visual disturbances.

Galactorrhoea with Hyperprolactinaemia -

Since the report by Chiari over a century ago galactorrhoea has been noted to occur with a wide variety of endocrine and non-endocrine disorders. With the exception of the study by Tolis et al, previous reports have dealt with a limited number of cases. The development of a sensitive bioassay for prolactin in human blood and subsequently of radioimmunoassays led us to study patients with galactorrhoea.

Physiology of Lactation -

Before pregnancy initial development & differentiation of the breast take place under the influence of estrogens, progesterone and prolactins with other hormones probably having a permissive action. The dominant role of prolactin was emphasized by Lyons, Li and Johnson who showed that in hypophyectomized, gonadictomized, adrenalectomized rats, estrogens, progesterone and other steroids were ineffective in the absence of prolactin in inducing any breast development. When prolactin was added, full growth and differentiation were obtained, upto and including the inducting of the secretory response. During pregnancy, under the influence of increased circulating amounts of estrogen, progesterone, prolactin and probably also placental lactogen, marked further lobular and alveolar development of the breast takes place, with eventual milk formation in the alveoli. Active lactation, however, does not begin until after parturition, and appears to be triggered by the sudden

decline in circulating estrogens and progesterone caused by the expulsion of placenta. Thereafter, lactation continues in an environment of low circulatory estrogen but relatively high prolactin, the latter hormone being actively stimulated by each sucking episode.

The role of estrogen is complex and appears to be at least three-folds: it stimulates the normal pituitary to secrete prolactin, it synergizes with prolactin in promoting breast differentiation, but in high concentration, it antagonizes prolactin at the level of the breast by inhibiting milk secretion. This action of estrogen is exploited for clinical purposes to suppress lactation in post-partum women who do not wish to nurse. In nursing mothers, prolactin levels decline gradually with increasing time post-partum, so that after several weeks to months, they may be within the normal range of non-nursing women. The act of suckling continues to produce some prolactin rise in most women even after prolonged periods post-partum. Thus for the initiation of normal lactation, high levels of prolactin appear to be necessary acting on a breast already primed by estrogen, progesterone and perhaps other hormones; once initiated, lactation may proceed with levels of prolactin not greatly different from those in the normal non-post-partum women.

Galactorrhea has been as an occasional accompaniment of acromegaly in the past, Davidoff having observed a

4 percent presence of galactorrhea in his series of 100 patients. David L. have also reported that elevated serum prolactin is found in approximately 40 per cent of patients with acromegaly. In the patients with galactorrhea and acromegaly who have normal serum prolactin, it may well be that the growth hormone exerts a lactogenic effect, since previous studies have shown that human growth hormone is almost as potent as prolactin when tested for lactogenic activity in mammalian bioassay. The coexistence of Cushing's Syndrome, galactorrhea and pituitary tumor has previously been reported, it seems likely that occasional tumors have the potentiality for hypersecretion of ACTH as well as prolactin.

Forbes and her colleagues suggested over-production of prolactin by pituitary tumors as causing galactorrhoea, although it was many years before this suggestion could be confirmed by measurement of prolactin in human blood. At least three mechanisms could explain such over-production. First of all, a primary disorder in the hypothalamus, involving diminished secretion of prolactin-inhibiting factor or over-production of a prolactin-releasing factor, could act on the pituitary to cause prolactin hypersecretion and possibly eventual tumor development. In the absence of adequate assays for these hypothalamic factors in blood or other fluids, such a possibility must remain speculative. Secondly, a pituitary tumor composed of non-functional

cells could impinge on the hypothalamus or its connections in such a way as to diminish the secretion of prolactin-inhibiting factor, leading to over-production of prolactin by normal, non-tumorous cells within the pituitary. Such a possibility seems unlikely on anatomic grounds in most cases, since adenomas, when found, may be relatively small, not extending above the diaphragma sellae. Further evidence against such a mechanism has been provided by immunocyto-chemical studies, which have shown large amounts of prolactin within the tumour cells and, in some cases, relatively little in adjacent non-tumor cells. The third possibility is autonomous secretion of prolactin by a tumor that is partly or entirely free of hypothalamic control. The high proportion of abnormalities on stimulation and suppression tests, together with the lack of normal 24 hours variation of prolactin in the one patient so tested, is consistent with this hypothesis, it does not rule out a primary hypothalamic origin, however.

It should be noted that over-production of prolactin by a pituitary adenoma is not always accompanied by galactorrhea. When prior estrogenic and progestational priming has been lacking, as in men, high prolactin levels alone are usually insufficient to cause milk secretion. The failure of galactorrhea to occur in some women with hyper-prolactinaemia may be related to inadequacy of prior steroidal priming, lack of an appropriate initiating event or other causes that are unclear.

Galactorrhea in the presence of regular menses may be commoner than is generally supposed was suggested by Nyir-jesy and Friedman & Gellfien. The absence of an obvious endocrine abnormality in most of these patients, reflected by the presence of menses and the generally normal serum prolactin levels, is further emphasized by the normal 24-hour prolactin studies and the normal response of most to thyrotropin-releasing hormone and L-dopa. The fact showed a subnormal response to chlorpromazine, however, suggests that despite the absence of demonstrable hyperprolactinaemia, a subtle abnormality of hypothalamic control may exist in a number of these patients.

Of importance is the fact that in two-thirds of the cases, galactorrhea began with parturition and persisted thereafter despite the resumption of menses. The physiology in these patients may thus resemble that of women many months post-partum who have continued to lactate because of nursing, but whose base-line serum prolactin concentrations are within the normal range. In both conditions the breasts have apparently become adopted to secrete milk with relatively low levels of prolactin stimulation. Some degree of prolactin still seems to be required, however, for galactorrhea has been reported to stop in such patients when the normal levels of prolactin are further reduced by the administration of ergot derivatives. From a practical standpoint, the finding of regular menses, normal skull films

and normal serum prolactin in a patient with galactorrhea makes the possibility of a clinically important pituitary tumor unlikely. Nevertheless, David, L. et al recommended that such patients continue to have prolactin determinations once a year and tomograms at intervals of three to five years, or more frequently if prolactin becomes elevated.

Argonz and Del Castillo described a group of patients who had amenorrhea together with galactorrhea for which no cause or association could be found. David, L. et al also described same group of patients.

Few patients had persistent galactorrhea and amenorrhea beginning after child-birth, and hence were classified as having the chiari-frommel syndrome. The nature of the hypothalamic pituitary disturbance that is brought out or caused by pregnancy in these patients is still unclear. That pituitary tumour may develop in a few patients initially considered have the Chiari-Frommel Syndrome has been suggested by Young et al, but such a progression not observed in any of our patients.

Galactorrhea has been reported to occur with the use or discontinuation of oral contraceptives, though the mechanism involved are uncertain. The withdrawal of estrogens after a period of stimulation may serve to trigger the onset of galactorrhea in a manner similar to the initiation of lactation after normal delivery, despite the lower levels

of hormonal stimulation. The existence of occult pituitary tumors in some of these patients is not unlikely.

Phenothiazines and other agents that antagonize dopaminergic pathways have been noted to cause galactorrhea in some patients. Hooper and his colleagues found that 26 percent of female psychiatric patients on high doses of these drugs had galactorrhea on testing by physical examination. The mechanism appears to involve reduction of hypothalamic secretion of prolactin-inhibition factor with consequent elevation of prolactin. David,L. et al reported that the majority of patients on chronic phenothiazine therapy have only moderate elevations of prolactin.

Galactorrhea has been noted as an uncommon accompaniment of primary hypothyroidism.

Few patients with galactorrhea and an enlarged sella turcica were demonstrated to have the empty-sella syndrome by pneumoencephalography or computerized axial tomography.

Treatment of Hyperprolactinaemia :

The presence of pituitary tumor in a woman in whom pregnancy was initiated by ovulation-induction is not necessarily an indication for tumour treatment during pregnancy. Most pregnancies can be successfully managed expectantly (Marshal, 1980). However, the patient should be followed carefully, with specific attention to the

development of headache and any visual disturbances which signify tumour enlargement during pregnancy. In general, if the patient remains asymptomatic, uneventful course of pregnancy and labour should be awaited.

1. Gonadotrophins -

Before bromocriptine became available human gonadotrophins were used to induce ovulation in most anovulatory women with hyperprolactinaemia, since these patients rarely ovulate after clomiptone treatment. Gonadotrophin treatment, on the other hand, proved to be very effective in women with amenorrhoea and galactorrhoea. A pregnancy rate of 80% was obtained by Rabau et al in 30 women with this syndrome.

The mean dose of human gonadotrophins in patients with post-partum amenorrhoea and galactorrhoea was not greatly different from that in the other anovulatory patients, and the mean duration of treatment was even shorter than that of amenorrhoeic women with lack of endogenous oestrogen activity. There is no evidence of ovarian resistance to exogenous gonadotrophins in amenorrhoeic women with hyperprolactinaemia. Nevertheless, human gonadotrophin treatment has considerable disadvantages and serous complications may occur.

2. Bromocriptin -

By virtue of the anti-mitotic or anti-proliferative action on the pituitary lactotrophs (Corenblum et al, 1975;

Corunblum, 1979; Marshal, 1980; Corenblum and Hanley, 1981 and Roon, 1981) bromocriptine is probably the best treatment for symptomatic enlargement of pituitary adenoma during pregnancy.

Although the traditional alternative for treatment of pituitary tumours have been radiotherapy or surgery or both (Thorner, 1977 and Husami et al, 1977), subsequently patients with microadenomas (10 mm or less of tumour diameter) were considered to be safe. Candidates for ovulation induction with bromocriptine (Gemzell and Wang, 1979 and Corenblum, 1979). Because of the absence of complications and the better acceptability of the pregnant women, and since complete or partial tumour regression has been extensively reported on non-pregnant patients (Thorner et al, 1980 and Corenblum, 1981), primary tumour treatment with bromocriptine appears at least an attractive alternative and perhaps even the treatment of choice for both micro and macro-adenomas (irrespective of tumour size) (Roon et al,1981 and Eanales et al, 1981).

The association of galactorrhoea with disorders of ovulation has been recognized for many years, but the presence of hyperprolactinaemia in some patients with such disorders has only recently been demonstrated (Jaffe et al, 1973; Seppala et al, 1975; Pepperell et al, 1976). Suppression of the elevated serum prolactin levels with bromocriptine (CB 154) has resulted in ovulation in many of these patients (Bessert et al, 1972; del Pozo et al,1974;

Tyson et al, 1975) but little is known of the usefulness of this drug in women with disorders of ovulation in the absence of hyperprolactinaemia.

The introduction of bromocriptine has dramatically changed the outlook for infertile women with hyperprolactinaemia, allowing fertility to be easily and effectively restored. Most of our hyperprolactinaemic patients with pituitary tumours also responded promptly to bromocriptin and ovulation can be induced even in patients with severe gonadotrophins deficiency and large pituitary tumours with high doses of bromocriptine. Nevertheless, if they become pregnant these patients are at a definite risk of developing serious complications from rapid tumour enlargement. Bergh,T. et al (1978) described a case who developed extensive visual field defects during third trimester of her bromocriptine-induced pregnancy. Corbey et al described another hyperprolactinaemic patient with a large pituitary tumour who developed progressive visual field defects from the 29th week of a bromocriptine-induced pregnancy.

Experimental studies have shown bromocriptine to be capable of reducing mitotic activity and inhibiting oestrogen induced proliferation of the pituitary and dopamine agonists can inhibit growth of pituitary tumours in rats, and even cause tumour regression. Bromocriptine is non-teratogenic. Children have born after bromocriptine-induced pregnancies without increased incidence of malformations.

Bergh, T. et al (1978) described that infertile patients with prolactin-secreting pituitary adenomas could be thoroughly informed of the risk of tumour complications during pregnancy and of the advantages and disadvantages of current forms of treatment. Some patients should perhaps be allowed to choose treatment with bromocryptine alone, provided that careful monitoring with frequent clinical examinations and visual field determinations is performed during the pregnancy. If visual complications still occur reinstitution of bromocryptine might be the primary treatment of choice. If this treatment fails alternative treatment as described above, have proved successful in preventing irreversible visual impairment.

Resistant to bromocryptine treatment results from deficient dopaminergic regulatory mechanisms in adenomatous cells. A decrease in D_2 dopamine receptor appears to be the main anomaly, but this phenomenon also might be associated with a post-receptor defect.

Lobo (1990) has suggested that tumor resistance to bromocryptine may be due to the fact that the tumor may not be a prolactin-secreting adenoma that is being treated although the prolactin level may be elevated and secondly prolactin may be immunologically aberrant e.g. big-big.

With normal basal prolactin values who were already ovulating before the bromocryptine was given, the urinary oestrogen and pregnanediol values were unchanged during the

treatment cycles compared with the cycles without bromocryptine. This indicates that bromocryptine itself has little direct effect on the ovaries, and that it acts entirely through the suppression of prolactin output (Pepperell, R.J. et al, 1977). These findings are in agreement with those of Del Pozo et al (1975) who found no change in hormone output when bromocryptine was given to normally ovulating women with normal prolactin.

The almost simultaneous development of radio-immunoassay for measurement of serum prolactin levels and the release of anti-prolactin agent, bromocriptine, for the effective suppression of elevated prolactin levels have revolutionized the treatment of anovulatory infertility of hyperprolactinaemic origin (Archer et al, 1974; Corunblum et al, 1976; Wiebe et al, 1977; Vaidya and Allorkar, 1977; Chang, 1978; Gemzell and Wang, 1979; Corunblum et al, 1979; Vaughn and Hammond, 1980; Papperell, 1981; Canales et al, 1981 and Crosighani et al, 1982).

However, as many of these women are young, wish to become pregnant, and very likely to have normal ovaries, they are good candidates for ovulation induction and are quite frequently treated with ovulation inducing agents (Gemzell and Wang, 1979).

When fertility is desired in a patient with pituitary prolactin secreting adenoma, then the choice of therapy should be non-destructive to the otherwise normal

non-adenomatous pituitary, yet bring normalization of serum prolactin levels with restoration of normal ovarian function (Corunblum, 1979).

Furthermore, it is feared that any tumour expansion during pregnancy may produce neurological complaints such as visual field abnormalities or persistent headache (Pepperel, 1981).

The choice of therapy in patients wishing to become pregnant are controversial and very depending on local clinical experience and abilities. There are advocates of pituitary microsurgery (Chang et al, 1977; Husami et al, 1977; Jaquet et al, 1978; Post et al, 1979; Kramer, 1980; Landolt, 1981; and Woosely et al, 1982) or radiation therapy (Thorner, 1977). Before conception is allowed, whereas others believe that the use of prolactin-lowering drugs such as bromocryptine with careful observation is the treatment of choice (Corunblum et al, 1975; Nergh et al, 1978; Corunblum, 1979; Thorner et al, 1980; Canalis et al, 1981 and Corunblum, 1981).

Because the natural history of these adenomas is not known, nor the effects of pregnancy on these adenomas, many patients are merely being followed clinically without any intervention (Gemzell and Wang, 1979).

Reports on the use of the drug during pregnancy have shown that it does not interfere with the normal progress of pregnancy (Ranta et al, 1980) and does not carry

increased risk of congenital malformations (Griffith et al, 1978). However, many investigators stop bromocryptine therapy prior to delivery (Yuen, 1978).

From the point of prognostic value, the ladies with low serum prolactin levels have very poor response to clomiphene and cyetic hormonal therapy followed by clomiphene, as compared to the ladies with normal or high prolactin values (Dutta, B., Dutta, A., 1995).

3. Irradiation -

In infertile hyperprolactinaemic patients with radiological evidence of pituitary tumour, Besser et al advise external pituitary irradiation (4500 Rads) from a linear accelerator prevent tumour growth during pregnancy. Irradiation must usually be combined with bromocryptine treatment as reduction of raised prolactin concentrations is slow and often incomplete after radiotherapy. Pre-treatment with irradiation result in uneventful pregnancies in three of four hyperprolactinaemic patients with tumours. One patient developed temporary visual field loss to red during the 38th week of pregnancy. She had shown variable visual fields before pregnancy hence the change during pregnancy might have been unrelated to treatment. Labour was induced and the field loss disappeared after delivery. Lamberts et al described a similar case of visual field defects developing after irradiation during a bromocryptine induced pregnancy, and concluded that prior radiotherapy

may not prevent visual complications from tumour growth during pregnancy. Nevertheless, their patient had a moderate suprasellar extension before the pregnancy.

Internal irradiation with yttrium implants in pituitary tumours before ovulation-inducing treatment was recommended by Child et al, who described seven uneventful pregnancies in hyperprolactinaemic patients after such treatment. But long term follow-up results of earlier radiation treatment show that such treatment may result in serious complications, such as loss of vision, brain necrosis and the development of sarcoma. Modern irradiation techniques may prevent such complications, but long-term follow-up results of such treatment are not yet available.

4. Surgery -

Surgery is recommended as the primary treatment of the fertility associated with prolactin-secreting pituitary adenomas. Selective removal of the pituitary adenoma by the transphenoidal route seems to be the method of choice if there is no suprasellar extension of the tumour. In 1973 Hardy reported that galactorrhoea disappeared after surgery in 18 women with amenorrhoea and galactorrhoea, but only five of them resumed regular menstruation. Gomez et al described the outcome of transphenoidal microsurgery performed by Hardy on 10 hyperprolactinaemic women with suspected pituitary tumours : menstruation returned in six, and four of them conceived. In three of the patients, no

adenoma was found on surgery. Kleinberg et al described 15 patients with tumour who underwent operation by the transphenoidal route. Only two of them resumed menstruation and became pregnant. In contrast, 17 out of 20 hyperprolactinaemic patients with tumour recently reported to have resumed menstruation after sphenoidal microsurgical exploration of the sella. Nevertheless, only two previously infertile patients were reported to become pregnant. Ovulatory cycles returned after transphenoidal surgery in five out of eight hyperprolactinaemic patients described by Franks et al. Thus the results of selective sphenoidal removal of prolactin-secreting pituitary adenomas are variable, and no data on recurrence rate on long term follow-up are yet available. Post-operative hypopituitarism still to be a risk; one of the eight patients reported to have developed panhypopituitarism after surgery.

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MATERIAL AND METHODS

MATERIAL AND METHODS

Present study was carried out in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi, over a period of one year (1996 - 1997).

Patients were selected from the out patient department and ward of Department of Obstetrics and Gynaecology of M.L.B. Medical College & Hospital, Jhansi.

Categorization of cases :

Cases were studied into two groups :

1. Control group : The control group comprised all the cases attending our out-door or admitted in ward with normal menstrual cycles and fertility of reproductive age group. Total number of cases included in this group were fifty.

2. Study group : This group comprised of women with infertility (both primary and secondary) with - menstrual dysfunction in the form of : Oligomenorrhoea, amenorrhoea primary or secondary, menorrhages, Irregular menses - Anovulatory cycles, ovulatory cycles, polycystic ovarian disease, Galactorrhoea. Total number of cases included in this group were one hundred fifty.

Patients were clinically examined and investigated and were divided into two main group I and II.

Group I comprised of 102 cases,

Group II comprised of 48 cases.

Both groups were further divided into two sub-groups :

Group A : Patients without Galactorrhoea, comprised of 141 cases.

Group B : Patients with Galactorrhoea, comprised of 9 cases.

Any watery or milky secretion expressed considered as positive for Galactorrhoea, if fat globules could be demonstrated on a wet mount of secretion.

History :

A detailed history of each patient was taken followed by thorough general, systemic and local examination as follows :

1. Name, age, OPD No. were recorded.
2. Occupational status was considered in order to know socio-economic status of the patient.
3. Educational status of each patient was asked.
4. Period of married life.
5. Period of staying with the husband.
6. Infertility (primary or secondary) and how long.

7. Age of first menstrual period.

8. H/o regular or irregular and painful or painless cycle.

9. History suggestive of ovulation.

10. H/o - Menorrhagia,
    - Polymenorrhoea,
    - Polymenorrhagia,
    - Oligomenorrhoea,
    - Amenorrhoea - Primary,
      - Secondary.

    Duration of menstrual dysfunction.

11. Coital frequency.

12. H/o sexual dysfunction : dyspareunia, apareunia, failure to maintain erection, orgasmic failure, premature ejaculation, effluvium seminis, retrograde ejaculation.

13. H/o Abstinence from sexual relation around the time of ovulation due to ovulation pain or spotting per vaginum.

14. H/o tuberculosis or contact with tuberculosis.

15. History suggestive of genital tuberculosis.

16. H/o Dilatation and curettage or any other pelvic operation including operations for infertility such as laparoscopy, tubal reconstructive surgery.

17. H/o Diabetes mellitus, Hypothyroidism, Hyperthyroidism, Galactorrhoea, endometriosis.

18. History suggestive of pelvic inflammatory disease.

19. Duration of last child birth.

20. Period of weaning.

21. History suggestive of intracranial tumor in or around sella turcica (headache, visual disturbances, disturbances of pituitary function.

22. History suggestive of encephalitis, meningitis or postencephalitic Parkinsonism.

23. History suggestive of menopause - physiologic or premature.

24. History of polycystic ovarian disease.

25. History suggestive of ovarian tumor - functional or non-functional (fibroma, carcinoma, cystoma or dermoid cyst).

26. History suggestive of adrenocortical dysfunction (Addison's disease, Hypernephroma, Adrenocortical tumor).

27. H/o mechanical hyperstimulation of breasts and/or nipples.

28. H/o Thoracic or breast surgery (stimulation of the afferents of the milk let down reflex).

29. H/o Burns on the anterior chest wall (peripheral neurogenic stimulation).

30. H/o Herpes Zoster of the chest wall.

31. H/o Drug therapy : Oral contraceptives, phenothiazines, resorpine, meprobamate, theophylline, alphamethyl dopa, amphitamine etc.

32. H/o Liver disease (decreased metabolism of prolactin and/or sex steroids).

33. H/o Hirsutism.

34. H/o Dyspareunia (due to dryness of vagina and atrophy of vaginal mucosa secondary to hypo-estrogenism).

Examination :

General Examination :- Thorough general examination was done with special attention to -

- Height, weight.
- Pallor,
- B.P.
- Lymphadenopathy,
- Obesity,
- Temperature,
- Goitre,
- Clinical evidence of Hypo or Hyperthyroidism, Addison's disease, Acromegaly, Hypopituitarism, diabetes mellitus.
- Hirsutism,
- Stage of development of axillary and pubic hairs.
- Chest wall lesions of Herpes Zoster, thoracotomy scar, Scars of thoracic wall burns.
- Breast : demonstration of galactorrhoea, stage of development.

Systemic Examination -

- Visual field defects as judged by confrontation method.

- Neurological examination for intracranial tumors, meningitis or encephalitis induced damage, Parkinsonism.
- Hepatomegaly.
- Examination of cardiovascular system, Respiratory system.

Per vaginal examination : Noting particularly the presence of pelvic tenderness, forniceal masses fixity of the uterus, uterine size, shape, ovarian tumors.

Per speculum examination -

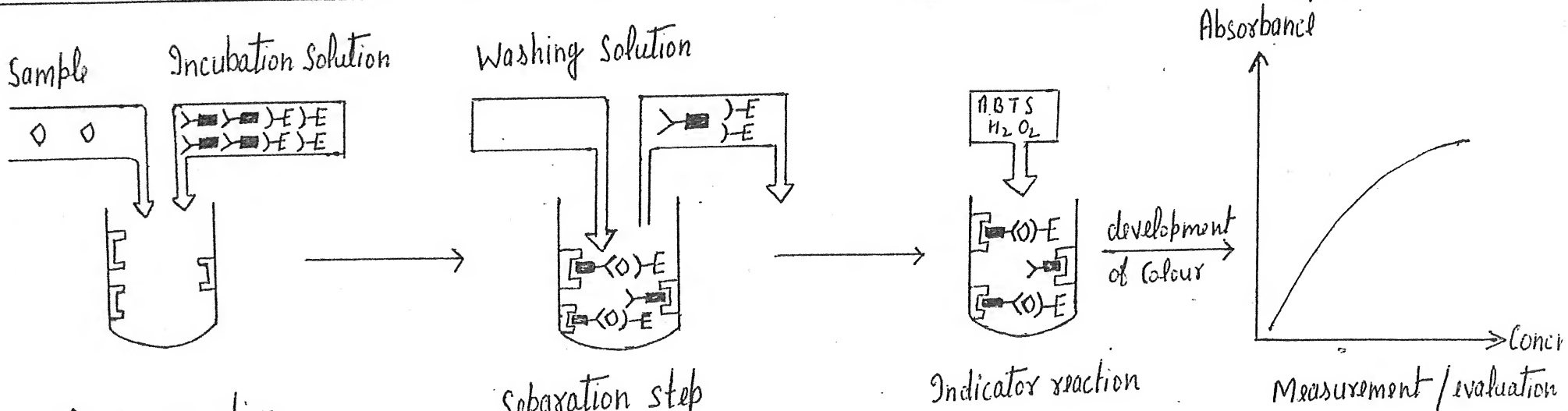
- Vaginal size : whether compatible with regular normal coitus.
- Evidence of cervical and/or vaginal infection.
- Cervical score, and whether the value is compatible with the day of the menstrual cycle.
- Dry and atrophic vagina (hypoestrogenism).
- Scanty cervical mucus (hypoestrogenism).

Investigations :

- Haemogram,
- TLC, DLC, ESR (for evidence of infection, tuberculosis).
- Blood sugar - fasting and postprandial.
- Urinalysis.
- Thyroid function test, adrenal cortical function test.
- Serial cervical scoring.

- BBT
- Husband's semen analysis
- Pos coital test,
- Laproscopy with chromopertubation and endometrial biopsy in the premenstrual phase.
- Hysteroscopy (for diagnosis of endometrial tuberculosis, endometrial synechiae, uterine septum). But it was not available in our department.
- Hysterosalpingography.
- Immunological studies : for anti-sperm antibodies in the serum of the patient and her husband and in the cervical mucus of the patient.
- Ultrasonography - ovulation study, PCOD,
- Gonadotrophins levels - FSH, LH.
- Serum Prolactin.
- Perimetry (for visual field defects),
- Radiograph of the skull, lateral view for pituitary tumour).
- High resolution CT Scan (for intracranial tumors). But it was not available in our department.
- Evaluation of ovarian mass.
- Evaluation of hirsutism.

TEST PRINCIPLE : ELISA / 1- Step Sandwich assay using Streptavidin technology



◊ Prolactin in sample

Streptavidin

Biotinylated anti-prolactin antibodies

POD - labelled antibodies

Serum Prolactin estimation :

Serum prolactin estimation was done by UBI. MAGIWEL™ PROLACTIN KIT or BOEHRINGER MANNHEIM PROLACTIN KIT. The UBI. MAGIWEL™ PROLACTIN QUANTITATIVE HP-201 is a solid phase enzyme linked immunosorbent assay. This test provides quantitative measurement of human prolactin in the serum.

Sample collection and handling : 3 ml of blood was withdrawn from anticubital vein of the patient subjected to following conditions :

1. She has fasted for 12-14 hours before such sample was taken and mid-morning sample was taken.
2. The blood was withdrawn without a minimal venous stasis with all aseptic precautions.
3. After withdrawing the sample, it was allowed to settle for ½ an hour facilitating the serum to separate, then centrifuged and serum was preserved with standard precautions at 2-8°C.
4. Avoid repeated freezing and thawing of serum specimens.

Preparation for assay :

- Before beginning the test, bring all samples and reagents to room temperature (20-25°C) and shake gently.
- Have all reagents and samples ready before the start of the assay. Once the test is begun it must be performed without any interruption to get the most reliable and consistent results.

- Use new disposable tips for each sample.

Assay Procedure -

- Secure the desired number of coated wells in the holder. Mark data sheet with sample identification.
- Dispense 25 ul of serum, standards or controls into appropriate wells.
- Dispense 100 ul of Enzyme conjugate into each well.
- Incubate for 30 minutes at room temperature.
- Rinse the wells 5 times with running tap water and flick the plate into sink to remove water.
- Dispense 100 ul of solution A and 100 ul of solution B into each well.
- Incubate for 10 minutes at room temperature.
- Stop reaction by adding 50 ul of IN H_2SO_4 to each well and read OD at 450 nm with a microwell reader.

Calculation of results -

Any microwell reader capable of determining absorbance at 450 nm may be used. The prolactin value of patient is obtained as follows :-

1. Plot the concentration of each Reference Standards (X-axis) against its absorbance (Y-axis) on graph paper.
2. Obtain the Prolactin values of samples by reference to the standard curve.

   Normal range for plasma prolactin is 1 to 27.5 ng/ml.

Other investigation in hyperprolactinaemic patients :-

- Plain X-ray of the skull with coned down views of pituitary fossa were obtained in patients with hyperprolactinaemia. A lesion of 1 cm in size was defined as macroadenoma and less than 1 cm as microadenoma.

- A thorough ophthalmological check-up was carried out especially visual field examination in patients who have hyperprolactinaemia.

- Medroxyprogesterone was administered to all the patients with amenorrhoea (5 mg TDS for 5 days) to assess the oestrogen status.

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OBSERVATIONS

OBSERVATIONS

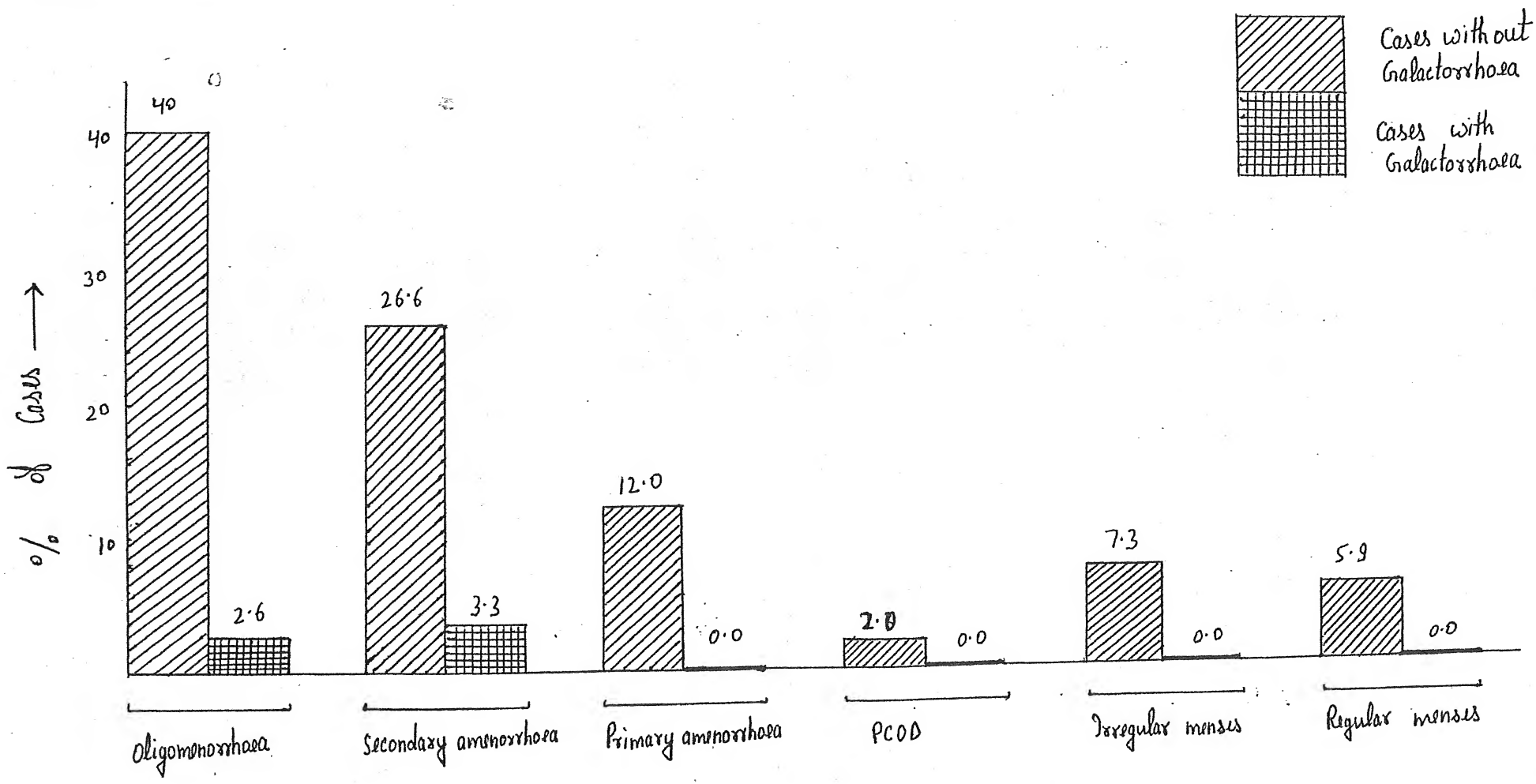
In the present study we have evaluated the serum prolactin assay in cases of primary and secondary infertility with menstrual dysfunction in the form of primary amenorrhoea, secondary amenorrhoea, oligomenorrhoea, irregular menses, regular anovulatory and ovulatory cycle, and polycystic ovarian disease cases. Cases were divided into two groups, with galactorrhoea and without galactorrhoea. Control group with normal fertility and normal menstrual history were selected for establishing the normal range of prolactin.

This study included one hundred fifty (150) cases and fifty (50) control group. Incidence of serum prolactin was studied in different groups and compared. Observed result of these cases are mentioned in various table forms.

Table - I

Showing the distribution of cases in various groups.

	No.of cases	Percentage
Cases :		
I. Primary infertility (age 20-35 years)	102	68.0
II. Secondary infertility (age 20-40 years)	48	32.0
Control cases (age 20-40 years)	50	-
Total	200	100.0



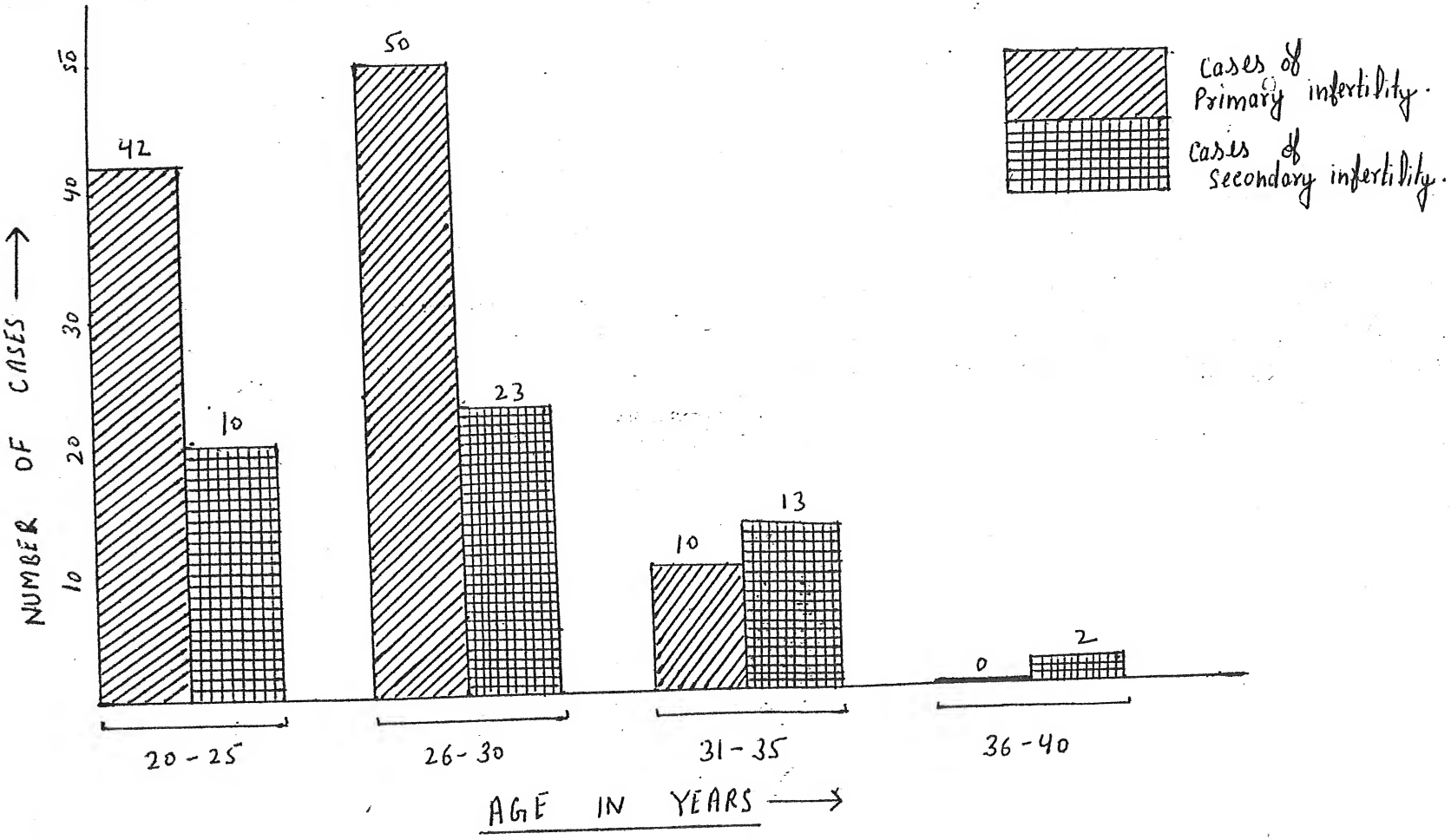
SHOWING THE DISTRIBUTION OF CASES IN VARIOUS GROUPS

Table - I showed the distribution of cases in control and study groups. Total number of primary infertility cases were 102 and secondary infertility were 48. Cases in control group were 50 only.

Table - II

Showing distribution of cases in various groups.

Group	Different groups of infertile patients	Total No.of cases	% of cases	Primary infertility(No.)	Secondary infertility(No.)
A	Without Galactorrhoea	141			
	1. Oligomenorrhoea	60	40.0	46	14
	2. Secondary amenorrhoea	40	26.6	18	22
	3. Primary amenorrhoea	18	12.0	18	-
	4. PCOD	3	2.0	3	-
	5. Irregular menses	11	7.3	6	5
	6. Regular anovulatory cycle	4	2.6	3	1
	7. Regular ovulatory cycle	5	3.3	4	1
B	With Galactorrhoea	9	6.0		
	1. Oligomenorrhoea	4	2.6	2	2
	2. Secondary amenorrhoea	5	3.3	2	3
	3. Primary amenorrhoea	-	-	-	-
	Total	150		102	48



SHOWING THE DISTRIBUTION OF INFERTILE PATIENTS ACCORDING TO THE AGE

Table - II revealed distribution of cases in -

Group A : Infertile patients without galactorrhoea and with different menstrual dysfunctions.

Group B : Infertile patients with galactorrhoea and different menstrual dysfunction.

Table - III

Showing the distribution of infertile patients according to the age.

Sl. No.	Age group	Number of patients			
		Secondary infertility		Primary infertility	
		No.	%	No.	%
1.	20 - 25	10	20.80	42	41.17
2.	26 - 30	23	47.90	50	49.08
3.	31 - 35	13	27.08	10	9.80
4.	36 - 40	2	4.22	0	-
	Total	48	100.00	102	100.00

Table - III showed distribution of cases according to age of patients. Majority of cases (49.08%) of primary infertility patients were in 26-30 years age group, followed by 20-25 years age group. Majority of cases (47.90%) of secondary infertility patients were in 26-30 years age group, followed by 27.08% in 31-35 years age group.

Table - IV(a)

Showing the distribution of primary infertility cases according to the marital period.

Years	2 - 5	6 - 10	11 - 15
No. of patients	80	17	5

Table IV(a) revealed distribution of primary infertility cases according to their marital period. Majority of patients (80 cases) had marital period of 2 - 5 years, followed by 17 cases of 6 - 10 years.

Table - IV(b)

Showing the distribution of secondary infertility cases according to period of last child birth.

No. of patients	10	30	8
Period of LCB (years)	3 - 5	6 - 8	9 - 11

Table - IV(b) observed that majority of secondary infertility cases (30) had period of LCB of 6 - 8 years, followed by 10 cases of 3 - 5 years.

Table - V

Showing the level of prolactin in control group.

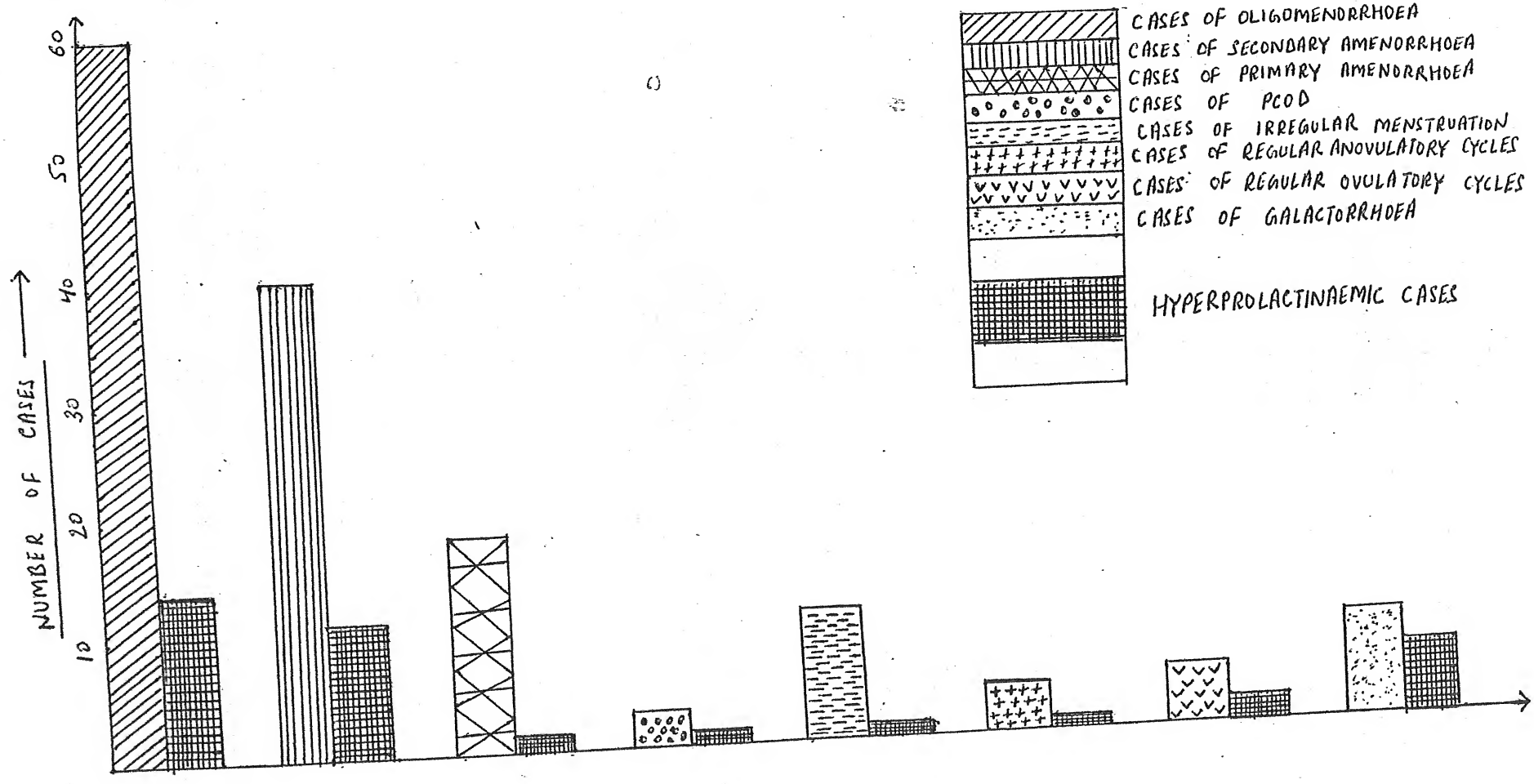
No.of cases	Range of prolactin levels in ng/ml	Mean ± S.D.
50	5.5 - 22.0	14.62 ± 4.31

Table V showed level of prolactin in control group. Lower limit was 5.5 ng/ml and maximum level was 22.0 ng/ml. Mean level was 14.62 ng/ml.

Table - VI(a)

Showing the prolactin concentration in different groups of infertile patients and incidence of hyperprolactinaemia.

Different groups	No.of cases	Prolactin level range ng/ml	Mean ±S.D.	Hyperprolactinaemia No.of cases	Hyperprolactinaemia %
A Without Galactorrhoea					
1. Oligomenorrhoea	60	5.0-105.0	29.92 ±23.95	14	23.34
2. Secondary amenorrhoea	40	7.5-180.0	39.63 ±43.81	11	27.50
3. Primary amenorrhoea	18	8.5-90.0	20.07 ±17.77	1	5.50
4. PCOD	3	22.0-35.5	27.34 ± 7.18	1	33.33
5. Irregular menstruation	11	12.0-90.0	25.67 ±21.56	1	9.09
6. Regular anovulatory cycles	4	16.5-362.0	10.50 ±13.08	1	25.00
7. Regular ovulatory cycles	5	5.5-60.5	29.90 ±23.33	2	40.00
N With Galactorrhoea	9	20.5-385.0	107.94 ±113.14	6	66.66
Total	150			37	



SHOWING THE DISTRIBUTION OF HYPERPROLACTINAEMIC CASES IN DIFFERENT GROUPS OF PATIENTS

Table - VI(a) showed majority of cases (66.66%) of hyperprolactinaemia were present in infertile cases with galactorrhoea. In cases without galactorrhoea, majority of cases (40%) were present in infertile cases with regular ovulatory cycles, followed by 33.3% cases with polycystic ovarian disease, and the minimum (5.5%) cases with primary amenorrhoea. Out of 150 infertile cases, hyperprolactinaemia was present in 37 cases i.e. 24.66%.

Table - VI(b)

Showing the menstrual pattern and serum prolactin level in hyperprolactinaemia.

Menstrual pattern	Mean serum PRO (ng/ml)	No. of patients (N=37)	%
1. Oligomenorrhoea	69.35	18	48.7
2. Secondary amenorrhoea	111.52	13	35.1
3. Primary amenorrhoea	90.00	1	2.7
4. Polycystic ovarian disease	35.50	1	2.7
5. Irregular menstruation	90.00	1	2.7
6. Regular anovulatory cycles	362.00	1	2.7
7. Regular ovulatory cycles	58.25	2	5.4

Table VI(b) revealed majority of hyperprolactinaemic patients have oligomenorrhoea, followed by secondary amenorrhoea. Mean serum prolactin level in patients with secondary amenorrhoea (111.52 ng/ml) was highly significant ($P < 0.001$) than that in patients with oligomenorrhoea. In regular anovulatory cycles, only one patient had higher prolactin level (362 ng/ml).

Table - VII

Showing the incidence of infertility in hyperprolactinaemia.

Infertility cases	Number of cases with hyperprolactinaemia	
	No.	%
Primary infertility	26	70.3
Secondary infertility	11	29.7
Total	37	100.0

Table VII showed majority of cases (70.3%) were of primary infertility in hyperprolactinaemic cases. Incidence of secondary infertility was 29.7% (11 cases). The test of proportion showed high significant difference ($P < 0.01$) between the two.

Table - VIII

Showing the incidence of galactorrhoea in hyperprolactinaemic cases.

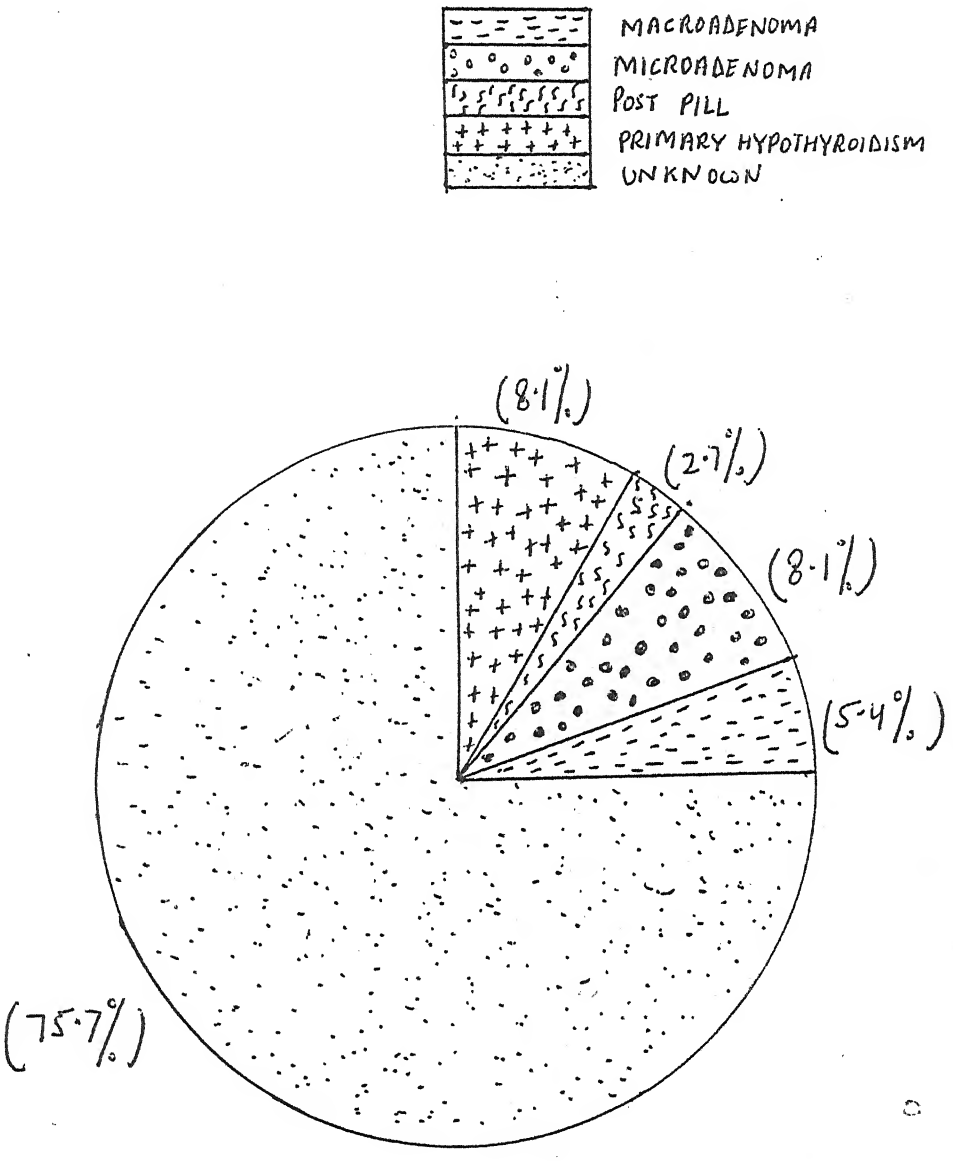
No.of cases having hyper-prolactinaemia	Cases with Galactorrhoea		Cases without Galactorrhoea	
	No.	%	No.	%
37	6	16.22	31	83.78
Mean serum PRO ng/ml	132.96		83.6	

Table VIII observed incidence of galactorrhoea was 16.22% in hyperprolactinaemic infertile cases. Galactorrhoea was not a constant feature in hyperprolactinaemia patients. High significant difference ($P < 0.01$) was observed between the mean serum prolactin of cases with galactorrhoea and without galactorrhoea.

Table - IX

Showing the hormonal profile in hyperprolactinaemic cases.

Diagnosis	No.of cases	Prolactin ng/ml		%
		Range	Mean ± S.D. (ng/ml)	
Macroadenoma	2	362-385	373.00±16.27	5.4
Microadenoma	3	105-180	144.34±37.63	8.1
Drug induced	-	-	-	-
Post Pill	1	28.5	-	2.7
Unknown	28	35-95.5	58.16±24.72	75.7
Primary Hypothyroidism	3	50.5-90	72.17±20.02	8.1
Total cases	37			100.0



DISTRIBUTION OF PATIENTS ACCORDING TO CAUSES OF HYPERPROLACTINAEMIA

Table IX showed incidence of etiology of hyperprolactinaemia on the basis of clinical diagnosis (history and examination), serum prolactin level, plain X-ray skull, later view, CAT scan was not available. Incidence of idiopathic hyperprolactinaemia was maximum in 28 cases (75.7%). No cases of drug induced hyperprolactinaemia was found in study group. Incidence of macroadenoma and microadenoma were 5.4% and 8.1% respectively. In macroadenoma, serum prolactin level was 360 - 385 ng/ml and in plain X-ray skull lesion was 7/1 cm size, and history of headache was present. In microadenoma, serum prolactin level was 105 - 180 ng/ml, and in plain X-ray skull lesion was /_1 cm size. In post pill hyperprolactinaemia, serum prolactin level was slightly higher than normal (28.5 ng/ml). In idiopathic cases, prolactin level was in between 35 - 65.5 ng/ml and in primary hypothyroidism 50.5 - 90 ng/ml.

Table - X

Showing the incidence of oestrogenised hyperprolactinaemic in different infertile cases.

No.of patients with hyperprolactinaemia	Oestrogenised		Poorly oestrogenised	
	No.	%	No.	%
Primary infertility (26 cases)	3	11.5	23	88.5
Secondary infertility (11 cases)	2	18.2	9	81.8
Total (37 cases)	5	13.8	32	86.2

Table - X revealed that majority of patients with hyperprolactinaemia on clinical basis were poorly oestrogenised supporting the assumption that prolactin exerts a negative peripheral effect on ovarian oestrogen production. Incidence of oestrogenised patients in primary infertility was 3 cases (11.5%) and in secondary infertility was 2 cases (18.2%).

Table - XI(a)

Showing serum prolactin levels in patients with macroadenoma, microadenoma and without adenoma.

Sl. No.	Serum prolactin level (ng/ml)	Macroadenoma (N=2)	Microadenoma (N=3)	No Adenoma (N=32)
1.	25 - 50	-	-	19
2.	51 - 100	-	-	13
3.	101 - 200	-	3	-
4.	201 - 300	-	-	-
5.	301 - 400	2	-	-
	Total	2	3	32

Table XI(a) reveals that serum prolactin in micro-adenoma was more than 100 ng/ml and less than 200 ng/ml. In macroadenoma it was more than 300 ng/ml. In cases without adenoma it was below 100 ng/ml.

Table - XI(b)

Showing the distribution of hyperprolactinaemic patients according to their menstrual dysfunction and suspected cause.

Menstrual dysfunction	Macroadenoma	Microadenoma	Post Pill	Unknown	Primary hyper-thyroidism
Without Galactorrhoea :					
• Oligomenorrhoea	-	-	1	11	2
• Secondary amenorrhoea	-	3	-	8	-
• Primary amenorrhoea	-	-	-	1	-
• Polycystic ovarian disease	-	-	-	1	-
• Irregular menses	-	-	-	-	1
• Regular anovulatory	1	-	-	-	-
• Regular ovulatory	-	-	-	2	-
With Galactorrhoea :					
• Oligomenorrhoea	-	-	-	4	-
• Secondary amenorrhoea	1	-	-	1	-
Total	2	3	1	28	3

Table - XI(b) shows incidence of adenoma was maximum in patients having amenorrhoea.

Table - XII(a)

Showing the effect of Bromocryptin use 5 - 7.5 mg/day orally in 15 cases for 1-6 months (N=15).

Sl. No.	Effect	Macroadenoma (N=2)		Microadenoma (N=3)		No adenoma (N=10)	
		No.	%	No.	%	No.	%
1.	Prolactin level come to normal	-	-	2	66.6	9	90.0
2.	Ovulation seen	-	-	2	66.6	9	90.0
3.	Conceive	-	-	1	33.3	5	50.0

Table - XII(a) shows that after the use of 5 - 7.5 mg/day Bromocryptin -

1) Macroadenoma cases - In no case, prolactin come to normal, ovulation not seen and none of them conceive. They need for R.T. or surgery.

2) In Microadenoma cases, two out of three have normal prolactin level, ovulation occur and one conceive.

3) In without adenoma cases - 9 out of 10 cases, prolactin come to normal, and ovulation occur and 5 cases conceive.

Table - XII(b)

Showing the effect of Bromocryptin use.

Sl. No.	Effect	No.of cases	Percentage
1.	Prolactin level come to normal	11	73.7
2.	Ovulation occur	11	73.7
3.	Conceive	6	40.0

Table - XII(b) shows that incidence of prolactin level come to normal was 73.7%, ovulation occurrence 73.7% and conception rate 40% after ruling out other infertility factors.

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D I S C U S S I O N

DISCUSSION

Classification and determination of aetiology of infertility in females have been based on various investigative procedures such as - clinical evaluation, endometrial biopsies, hysterosalpingography, endoscopy and endocrine studies. Menstrual dysfunction with infertility is a distinct clinical entity (d/t) derangement in function of the hypothalamo-pituitary ovarian - uterine axis, thereby resulting in absence of menstruation for a variable period. Knowledge of the basic gynaecological endocrinology has been of paramount significance in the investigative approach in cases of menstrual dysfunction with infertility. Recent advances in reproductive endocrinology has increminated the anterior pituitary polypeptide hormone prolactin in the pathogenesis of anovulation in amenorrhoea, galactorrhoea syndrome and other menstrual dysfunction with infertility.

A patient with hyperprolactinaemia presents with a broad spectrum of signs and symptoms. The importance of hyperprolactinaemia in menstrual disturbances and infertility is well recognized classical presentation described with hyperprolactinaemia in secondary amenorrhoea with or without galactorrhoea. In clinical practice, it is not uncommon to see a patient with hyperprolactinaemia presenting with regular

anovulatory cycles, oligomenorrhoea, primary amenorrhoea or luteal phase defect.

The present study was carried out to find out the incidence of hyperprolactinaemia in infertility with different menstrual disorders, and serum prolactin assay in cases of primary and secondary sterility with galactorrhoea or without galactorrhoea.

The present study included one hundred fifty cases and fifty in control group. Out of one hundred fifty cases, there were 102 cases (68%) of primary infertility and 48 cases (32%) of secondary infertility. Control group cases have normal fertility and normal menstrual cycles.

Age of control cases were 20-40 years. Primary infertility cases belonged to the age group of 20-35 years, and secondary infertility to the age group of 20-40 years. Majority of primary infertility (49.08%) and secondary infertility (47.9%) cases belonged to age group of 26-30 years.

Majority of secondary sterility cases had period of LCB of 6-8 years and majority of primary infertility patients had marital period of 2-5 years.

In our study, level of serum prolactin in control group was 5.5 - 22 ng/ml and mean level was 14.62 $\pm$ 4.31. All of them have level within normal limit. Our findings correlates with the findings of Sinha, G.(Mrs.) et al (1989).

In our study, incidence of hyperprolactinaemia was 24.66%. Our findings were similar to the findings of Jayesh J. Sheth & Frenny, J. Sheth (1992) who found incidence of hyperprolactinaemia 24.7%. In contrast, Sinha, G. et al (1989) who found incidence of hyperprolactinaemia was 13.3% in cases of infertility with menstrual disturbances and Pillai, V.N. et al (1991) found incidence of hyperprolactinaemia to be 5.48% in their infertility clinic.

De Pozo (1976) - It is therefore possible to affirm that the subjective response of female reproductive axis shows a larger degree of sensitivity to hyperprolactinaemia. So the measurement of serum prolactin levels alone is a definitive stage in the exclusion of such a syndrome in subjects with different menstrual disorders.

In our study, hyperprolactinaemia is present in 23.34% patients with oligomenorrhoea, 27.5% patients with secondary amenorrhoea, 5.5% patients with primary amenorrhoea, 33.3% patients with polycystic ovarian disease, 9.09% patients with irregular menstruation, 25% patients with regular anovulatory cycles, 40% patients with regular ovulatory cycles.

Hyperprolactinaemia is present in 22.8% (Pepperell 1978) to 33% (Seppala et al, 1977) of patients with secondary amenorrhoea, in 2 to 8 percent of patients with oligomenorrhoea and in 2.5% (Papperell, 1978) to 4% of patients with regular

cycles with unexplained infertility and corpus luteum defects. Thus our findings are concurrant with the above.

Sinha, G. et al (1989) reported 8.9% of hyperprolactinaemia in patients of oligomenorrhoea, 24.4% of hyperprolactinaemia in patients of secondary amenorrhoea. They did not find any case of hyperprolactinaemia in primary amenorrhoea and in luteal phase defect incidence of hyperprolactinaemia was 6.2%.

Jayesh J. Sheth (1992) found 30% patients with polycystic ovarian disease had hyperprolactinaemia. Our findings are consistent to the study made by them. Since chronic anovulation and disordered LH-FSH secretion appear to be basic features of polycystic ovarian disease, it is likely that endorphine liberation in the hypothalamus suppresses both dopamine and Gn RH pathways leading to hyperprolactinaemia. High prolactin levels also inhibit oestrogen synthesis in the ovary leading to chronic anovulation and polycystic ovarian disease.

Jayesh J. Sheth (1992) also found 27% of women with oligomenorrhoea have hyperprolactinaemia. This is because of increased prolactin levels produces anovulation and it prevents the LH pulsatility and interferes with the positive feedback action of estradiol at the hypothalamic level through blockage of the estrogenic receptors.

Similar to our findings, Jayesh J. Sheth (1992) found idiopathic hyperprolactinaemia (26.31%) in normally

menstruating women with ultrasonographically documented ovulation. 15% of patients had hyperprolactinaemia during pre-ovulatory phase and 26.31% of patients had hyper-prolactinaemia in mid-luteal phase. Hyperprolactinaemia in women with regular ovulatory menstrual cycles, has been observed by Isac and Kyogo (1985). Recently, Robert Harrison (1988) has reported stress spikes of hyperprolactinaemia in couples with unexplained infertility. Thus we conclude that all patients with sterility irrespective of menstrual disorders or ovulation should be screened for prolactin estimation. Pillai, V.N. et al (1991) found in their study that 70% of hyperprolactinaemic patients presented with regular menstrual cycles. In contrast to above study, in our study we found 8.1% regular menstrual cycles in hyperprolactinaemic patients.

In our study, out of 37 hyperprolactinaemic patients majority of patients (48.7%) had oligomenorrhoea, followed by secondary amenorrhoea (36.13%).

The mean serum prolactin level in patients with secondary amenorrhoea (262.5 ng/ml) was significantly higher than that in patients with oligomenorrhoea, irregular menstruation and regular anovulatory cycles (95.88 ng/ml) (Pillai, V.N. et al, 1991). Similar findings were reported in our study. In secondary amenorrhoea (111.52 ng/ml) was significantly higher than in patients with oligomenorrhoea (69.35 ng/ml), primary amenorrhoea (90 ng/ml) and irregular menstruation (90 ng/ml). These findings suggest that higher

the prolactin levels, the more significant will be menstrual disturbance. Similar findings were reported by Jayesh J.Sheth and Frenny J. Sheth (1992) i.e. serum prolactin levels were significantly raised in women with secondary amenorrhoea (104.2 ± 11.35 ng/ml).

In our study, incidence of hyperprolactinaemia in patients with galactorrhoea was 66.6% are in agreement with the view of Sinha, G. et al (1989), who found the incidence of 62.5%. Jayesh J. Sheth and Frenny J. Sheth (1992) also found the incidence of hyperprolactinaemia in patients with galactorrhoea was 44%. Serum prolactin level was significantly raised (132.96 ng/ml). Similar findings were reported by Jayesh J. Sheth and Frenny J. Sheth (1992) i.e. 75 ± 34.42 ng/ml).

Galactorrhoea was not a constant feature in hyperprolactinaemic patients. Our findings that only 16.22% of patients had demonstrable galactorrhoea is comparable to approximately 30% association seen in most of the series (Lawrence et al, 1983; Sinha et al, 1989) and 28% in Pillai, N. et al (1991). Del Pozo (1978) reported 30-40% patients with galactorrhoea had hyperprolactinaemia. However, it is also present in 10% of normoprolactinaemia amenorrhoea. A poor correlation between galactorrhoea and hyperprolactinaemia has been found in patients with normal menstrual function. Kleinberg et al (1977) has reported that about one-third of

women with galactorrhoea have normal menses. In our study, we did not find such correlation and patients of galactorrhoea had oligomenorrhoea and secondary amenorrhoea.

David L. Kleinberg et al (1977) found 8.5% patients had amenorrhoea together with galactorrhoea. In our study 3.3% of patients had both. These patients resemble those described by Argonz and Del Cartillo (1953). Mean prolactin in these patients was markedly elevated at 89.1 ng/ml (David L. Kleinberg et al, 1977), in our study it was 113.3 ng/ml.

9.8% patients in whom galactorrhoea appeared to be related to the taking oral contraceptive pills (David, L. Kleinberg, et al, 1977). In our study, 2.7% patients had post pill hyperprolactinaemia. Mean prolactin level was 49.6 ng/ml in their study and in our study it was 28.5 ng/ml. Similar findings of prolactin level in post pill amenorrhoea (25 - 30 ng/ml) were reported by Mohanty, S. et al (1993) which correlated with the observation of Khandelwal (1985).

David L. Kleinberg et al (1977) found 6.8% patients had galactorrhoea in association with drug therapy. In our study we did not find any correlation. Hooper and his colleagues found that 26% of female psychiatric patients on high doses of these drugs had galactorrhoea.

Moreover, the assay of prolactin in serum is undoubtedly one of the most useful assays for tumour related

antigens as the prolactinoma may be present in 25 to 34% of women with amenorrhoea and/or galactorrhoea and hyperprolactinaemia (Kleinberg et al, 1977).

Rajan, R. and Ambika, P. (1984) found incidence of tumour in hyperprolactinaemia with amenorrhoea was 30.7%. Similar incidence of tumour in patients with amenorrhoea and hyperprolactinaemia was between 36% (Frank et al, 1977) and 48% (Kleinberg et al, 1977). In our study we found similar incidence in these patients was 41.6%.

Pituitary tumour accounted for 12% cases of hyperprolactinaemia (Pillai, V.N. et al, 1991). In our study we found similar incidence 13.1%.

David L. Kleinberg et al (1977) found that patients with pituitary tumour had the highest serum prolactin concentration. The pituitary tumour was directly correlated with the height of the serum prolactin. Above 300 ng/ml, all prolactin concentrations in this series were associated with pituitary tumours, above 100 ng/ml, 57% of patients had tumours. Pillai, V.N. et al (1991) also found that patients with pituitary adenoma had a significantly higher baseline serum prolactin level (mean 271.6 ng/ml) than other causes (mean 65.22 ng/ml). Our findings are consistent to the study made by above given workers. In our study, two patients had mean serum prolactin 373 ng/ml were cases of macroadenoma and 3 cases had mean serum prolactin 144 ng/ml were cases of microadenoma.

Serum prolactin level was significantly elevated more than 150 ng/ml in cases of macroadenoma and 90 - 120 ng/ml in cases of microadenoma (Mohanty, S. et al, 1993). In our study, incidence of macroadenoma was 5.4%. Similar incidence 6.2% were reported by Sinha, J. et al (1989); 6% by Pillai, V.N. et al (1991).

Serum prolactin level is directly proportional to the size of prolactinoma. Hence, any patients with prolactin level greater than 1000 u IU/ml should be thoroughly investigated (Franks and Jacobs, 1983).

In our study, 20% of women with amenorrhoea and galactorrhoea (one out of 5 cases) had radiologically evident pituitary tumour and this patient had the highest serum prolactin concentration and 33.3% consisted of women with idiopathic galactorrhoea had normal prolactin level. Similar findings were reported by David L. Kleinberg et al (1977) in which they found 34% of women with amenorrhoea and galactorrhoea had radiologically evident pituitary tumours and these patients had the highest serum prolactin concentration and 32% consisted of women with idiopathic galactorrhoea with normal prolactin in 86% of these cases.

On clinical basis i.e. appearance of patient, vaginal examination, size of uterus, endometrial biopsy, our findings that majority of patients with hyperprolactinaemia were poorly oestrogenised. Only 13.8% patients were oestrogenised are

in agreement with the views of Sinha, G. et al (1989), only 12.5% patients oestrogenised. Similar findings were reported by Jacobs et al (1976).

In our study, out of 37 hyperprolactinaemia patients, 15 patients came for regular follow-up and received bromocryptine in dosage of 2.5 to 7.5 mg for a period ranging from 1-6 months. Prolactin level came to normal in 73.7% cases, ovulation occur in 73.7% cases and 40% cases conceived and delivered term babies. There were no congenital anomalies or abortion in bromocryptine induced pregnancy.

Dutta, B. et al (1995) found incidence of ovulation in hyperprolactinaemia patients was 41.6%, while in our study it was 73.7%, which was similar to the results obtained by Pepperell, R.J. et al (1977) who found 85% ovulation rate. Most of hyperprolactinaemic patients with pituitary tumours also responded promptly to bromocryptine and ovulation can be induced even in patients with severe gonadotrophins deficiency and large pituitary tumour with high doses of bromocryptine (Bergh, S. et al, 1978).

In the study of Pillai, V.N. et al (1991), corrected pregnancy rate was 14.3% and in David, L. Kleinberg et al (1977) it was 16.66%, but in our study it was more i.e. 40%. But it is less than the results obtained by Pepperell, R.J. et al (1977) who found 70% pregnancy rate. The better response of patients with elevated prolactin values has already been reported by Jacobs et al (1976), although Seppala et al (1976)

found an identical ovulation rate in hyperprolactinaemia patients and patients with normal prolactin.

Pregnancy rate of 80% was obtained by Rabau et al in women with amenorrhoea and galactorrhoea by human gonadotrophins use. The introduction of bromocryptine has dramatically changed the outlook for infertile women with hyperprolactinaemia and allowing fertility to be easily and effectively restored (Bergh, S. et al, 1978).

David, L. Kleinberg et al (1977) also reported that bromocryptine lowered prolactin in all patients with idiopathic hyperprolactinaemia or pituitary tumour stopped galactorrhoea in over 50%, restored menses in more than 70%.

In our study no teratogenic effects of bromocryptine has been reported. Similar findings were reported by Del Pozo (1976) and Pillai, V.N. (1991). Del Pozo (1976) reported there would appear to be no justification in continuing the drug once pregnancy has been confirmed.

In our study, there is no visual field defects during pregnancy. But in Bergh, S. et al (1978) study, this was shown in one of their patient who developed extensive visual field defects during third trimester of her bromocryptine-induced pregnancy. Corbey et al (1977) described another hyperprolactinaemic patient with a large pituitary tumour who developed progressive visual field defects from the 29th week of a bromocryptine-induced pregnancy.

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SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

The present study entitled "Serum prolactin assay in menstrual dysfunction with infertility" was conducted in the Department of Obstetrics and Gynaecology, M.L.B. Medical College, Jhansi. Cases were selected from the out patient department and ward of Department of Obstetrics & Gynaecology of M.L.B. Medical College & Hospital, Jhansi.

This study included 200 cases aged 20 years to 40 years of age. 150 women with infertility (both primary and secondary) with menstrual dysfunction in the form of oligomenorrhoea, secondary amenorrhoea, primary amenorrhoea, irregular menses, regular ovulatory or regular anovulatory menses and women with galactorrhoea & polycystic ovarian disease were taken as study group and 50 women with normal menstrual cycles and fertility were taken as control group to establish the normal range and mean serum prolactin. Serum prolactin was measured by radio-immune assay the BOEHRINGER MANNHEIM - PRL Kit or "UBI-MAGIWEL" - PRL Kit.

Following conclusions were drawn from this study :

1. Majority of primary infertility (49.08%) and secondary infertility (47.9%) cases belonged to age group of 26 - 30 years.

2. Majority of secondary sterility cases had period of last child birth of 6-8 years and majority of primary infertility patients had marital period of 2-5 years.

3. In our study, incidence of hyperprolactinaemia was 24.66%.

4. In our study, incidence of hyperprolactinaemia in different groups was -

Patients with oligomenorrhoea	=	23.34%
Patients with secondary amenorrhoea	=	27.5%
Patients with primary amenorrhoea	=	5.5%
Patients with polycystic ovarian disease	=	33.3%
Patients with irregular menstruation	=	9.09%
Patients with regular anovulatory cycles	=	25%
Patients with regular ovulatory cycles	=	40%.

5. In our study in hyperprolactinaemic patients majority of patients (48.7%) had oligomenorrhoea, followed by secondary amenorrhoea (35.13%).

6. The mean serum prolactin level in patients with secondary amenorrhoea (111.52 ng/ml) was significantly higher than in patients with oligomenorrhoea (69.35 ng/ml), primary amenorrhoea 90 ng/ml and irregular menstruation (90 ng/ml). These findings suggest that higher the prolactin levels, the more significant will be menstrual disturbance.

7. In our study, incidence of hyperprolactinaemia in patients with galactorrhoea was 66.6%. Serum prolactin level was significantly raised (132.96 ng/ml) in these cases.

8. Galactorrhoea was not a constant feature in hyperprolactinaemic patients. In our study in hyperprolactinaemic patients, only 16.22% had demonstrable galactorrhoea.

9. In our study, 2.7% patients had post pill hyperprolactinaemia and serum prolactin was 27.5 ng/ml.

10. In our study we did not find any drug induced hyperprolactinaemia.

11. Pituitary tumour accounted for 13.1% cases of hyperprolactinaemia. Moreover, the assay of prolactin in serum is undoubtedly one of the most useful assays for tumour related antigens.

12. Incidence of tumour in patients with hyperprolactinaemia with amenorrhoea was 41.6%.

13. Pituitary tumour was directly correlated with the height of the serum prolactin. In our study, two patients had mean serum prolactin 373 ng/ml were cases of macroadenoma on X-ray skull, and 3 cases had mean serum prolactin 144 ng/ml were cases of suspected microadenoma.

14. In our study, majority of patients with hyperprolactinaemia were poorly oestrogenised.

15. In our study, incidence of macroadenoma was 5.4%.

16. In our study, out of 37 hyperprolactinaemic patients, 15 patients came for regular follow-up and received bromocryptine in dosage of 2.5 to 7.5 mg/day for a period ranging from 1-6 months. We found prolactin level come to normal in 73.7% cases, ovulation occur in 73.7% cases, and 40% cases conceived and delivered term babies. There were no congenital anomalies or abortion in bromocryptine induced pregnancy and no visual field defects develop during pregnancy.

Hyperprolactinaemia is quite an important cause of infertility and menstrual dysfunction. Galactorrhoea, its typical clinical marker is not present in all the patients. Hence, serum prolactin estimation is mandatory for the diagnosis. Specific treatment of this condition can be instituted with bromocryptine after confirmation of diagnosis.

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A P P E N D I X

MASTER CHART

Patients of Primary infertility with Oligomenorrhoea.

Sl. No.	Name	Age (yrs.)	Wt. (kg.)	Married period (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml.)
									Vagina	Uterus	
1.	Mamta	26	55	4	Absent	Feminine	No	Absent	Normal estrogenised	Normal size	20.0
2.	Asha Singh	35	60	15	Absent	Feminine	No	Absent	Estrogenised	Normal size	12.5
3.	Radhika	28	52	4	Absent	Feminine	No	Absent	Poorly oestrogenised.	Small size	65
4.	Sangeeta	22	55	3	Absent	Feminine	No	Absent	Oestrogenised.	M.P.S.	5.0
5.	Divya	24	56	2	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	50
6.	Archna	22	59	2	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	20
7.	Kavita	28	52	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	27.5
8.	Shanti	21	55	3	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	12
9.	Suman	23	56	2	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	18
10.	Sabana	25	52	2	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	23
11.	Vijaya	25	53	3	Absent	Feminine	No	Absent	Oestrogenised.	Small size.	16
12.	Sita	25	55	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	19
13.	Vidhya	32	50	10	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	20
14.	Vaishali	28	51	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	6
15.	Chanda	31	56	8	Absent	Feminine	No	Absent	Oestrogneised.	Small size.	16
26.	Kiran	27	52	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	17
17.	Geeta	28	48	3	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	18
18.	Raj Kumari	30	62	2	Absent	Feminine	No	Absent	Poorly oestrogenised.	Small size.	60
19.	Gayatri	21	52	2	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	18
20.	Gomti	28	56	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	20
21.	Bhagwati	32	46	6	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	22
22.	Archna	31	45	9	Mild headache.	Feminine	No	Absent	Poorly oestrogenised.	Small size	90

Patients of Primary infertility with Oligomenorrhoea (Contd...)

Sl. No.	Name	Age (yrs.)	Wt. (kg.)	Married period (yrs.)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
23.	Guddi	26	52	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	21.5
24.	Jai Kunwar	22	46	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size.	19
25.	Urmila	22	53	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size.	19
26.	Uma	26	54	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size.	8
27.	Lad Kunwar	28	52	6	Headache.	Feminine	No	Absent	Poorly oestrogensd.	Small size.	102
28.	Sufiya	26	54	6	No	Feminine	No	Absent	Poorly oestrognsd.	Small size.	65.5
29.	Usha	20	50	2	No	Feminine	No	Absent	Oestrogenised.	Normal size.	23
30.	Sangeeta	30	51	9	No	Feminine	No	Absent	Oestrogenised.	Normal size.	20
31.	Madhu Sharma	26	46	4	mild headache.	Feminine	No	Absent	Poorly oestrognsd.	Small size.	84
32.	Manju	20	44	2	No	Feminine	No	Absent	Oestrogenised.	Normal size.	23
33.	Ram murti	22	48	3	No	Feminine	No	Absent	Oestrogenised	Normal size.	15.5
34.	Kalpana	26	50	4	No	Feminine	No	Absent	Oestrogenised	Normal size.	12
35.	Ramjanki	27	51	4	No	Feminine	No	Absent	Oestrogenised	Normal size.	20
36.	Shakila	32	59	10	No	Feminine	No	Absent	Oestrogenised	Normal size.	15.5
37.	Sushma	25	57	3	No	Feminine	No	Absent	Oestrogenised	Normal size.	20
38.	Bhuri	28	52	6	No	Feminine	No	Absent	Oestrogenised	Small size.	22
39.	Mithlesh	35	50	14	No	Feminine	No	Absent	Oestrogenised	Normal size.	19
40.	Vimla Raj	23	58	3	No	Feminine	No	Absent	Oestrogenised	Normal size	15
41.	Babli	22	49	3	No	Feminine	No	Absent	Oestrogenised	Normal size.	7.6
42.	Manjulata	28	61	8	No	Feminine	No	Absent	Oestrogenised	Normal size.	20
43.	Parwati	26	65	2	No	Feminine	No	Absent	Oestrogenised	Normal size.	8.5
44.	Kalpana	31	59	12	No	Feminine	No	Absent	Oestrogenised	Normal size	58.5
45.	Satyakali	23	52	4	No	Feminine	No	Absent	Oestrogenised	Normal size.	15
56.	Anjana	27	54	4	No	Feminine	No	Absent	Oestrogenised	Normal size.	18
47.	Archna	28	56	3	No	Feminine	No	Present B/L +	Poorly oestrogenised.	Small size.	85.5
48.	Vimla	24	52	2	No	Feminine	No	Present B/L ++	Poorly oestrogenised.	Small size.	79.8

Patients of Secondary Infertility with Oligomenorrhoea.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	LCB (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Kanaklata	35	46	10	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16
2.	Alka	24	48	5	Absent	Feminine	No	Absent	Oestrogenised	Normal size	5.0
3.	Sangeeta	33	52	8	Absent	Feminine	No	Absent	Poorly oestrogenised	Small size.	38.5
4.	Sunita	28	59	6	Absent	Feminine	OCP	Absent	Oestrogenised	Normal size	28.5
5.	Sharda	25	64	6	Absent	Feminine	No	Absent	Oestrogenised.	Normal size	18
6.	Manju	27	48	7	Absent	Feminine	No	Absent	Poorly oestrogenised	Small size	36
7.	Asha Verma	32	60	4	Absent	Feminine	No	Absent	Oestrogenised.	Normal size.	6.0
8.	Ram Kunwar	30	54	8	Absent	Feminine	No	Absent	Poorly oestrogenised.	Small size	64
9.	Laxmi	29	53	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	15
10.	Archna	24	57	5	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Normal size	76
11.	Babli	33	52	9	Absent	Feminine	No	Absent	Oestrogenised	Normal size	18
12.	Pushpa	25	46	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	12.0
13.	Ramdevi	30	44	7	Headache	Feminine	No	Absent.	Poorly oestrogenised.	Small size	100
14.	Gayatri	33	61	5	Absent	Feminine	No	Absent	Oestrogenised	Normal size	13.5
15.	Uma	25	58	6	Headache	Feminine	No	Present B/L ++	Poorly oestrogenised.	Small size	95.2
16.	Pushpa	30	52	8	Absent	Feminine	No	Present. B/L +	Poorly oestrogenised.	Small size	86.5

Patients of Primary infertility with secondary amenorrhoea.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	Married period	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Rajni	21	52	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	17
2.	Ramkali	22	54	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	7.5
3.	Usha	27	59	4	Headache	Feminine	No	Absent.	Poorly oestrogenised.	Small size.	180
4.	Somwati	26	48	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20
5.	Anjali	20	45	2	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size.	68
6.	Rani	26	49	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22
7.	Kusum	27	48	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.5
8.	Aneeta	23	56	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	42.5
9.	Mamta	27	52	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	10.5
10.	Laxmi	28	61	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	17
11.	Sunita	29	66	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20
12.	Sudha	27	52	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	23
13.	Sangeeta	24	54	2	Absent	Feminine	No	Absent.	Poorlyoestrogenised.	Small size	83.5
14.	Bhagwati	28	52	4	Headache	Feminine	No	Absent.	Poorly oestrogenised.	Small size.	124
15.	Abhilasha	27	61	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	24
16.	Dhara	27	58	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22
17.	Rekha	25	46	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	8.0
18.	Sunita	31	49	11	Absent	Feminine	No	Absent	Oestrogenised	Normal size	15.0
19.	Anita	29	52	4	Absent	Feminine	No	Present B/L +	Oestrogenised	Normal size	24.5
20.	Mumtaj	27	54	12	Absent	Feminine	No	Present U/L +	Poorly oestrogenised.	Small size	22.0

Patients of secondary infertility with secondary amenorrhoea.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	LCB (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Guddi	29	46	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	12
2.	Jyoti	34	48	10	Absent	Feminine	No	Absent	Oestrogenised	Normal size	10
3.	Rajwati	24	56	6	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size.	100.5
4.	Neena	28	59	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.5
5.	Anita	27	61	5	Absent	Feminine	No	Absent	Oestrogenised	Normal size	19
6.	Sangeeta	33	60	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	23
7.	Kuljeet Kaur	30	52	7	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size	76
8.	Sushma	28	54	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	7.5
9.	Sayara	25	48	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22
10.	Salma	28	49	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	10
11.	Sunita	35	50	11	Headache	Feminine	No	Absent.	Poorly oestrogenised.	Small size.	148
12.	Sushma	37	56	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22
13.	Rekha Dube	26	52	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20
14.	Munni	27	55	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	42
15.	Alka	25	49	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	12
16.	Savitri	28	44	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.5
17.	Sunita	35	48	10	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size	58
18.	Mamta	30	61	6	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size	76.5
19.	Priti	24	62	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.5
20.	Sharda	30	52	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	13
21.	Babita	33	48	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	19
22.	Kranti	23	46	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	18
23.	Rajeshwari	27	56	4	Headache	Feminine	No	Present, B/L +++	Poorly oestrogenised.	Small size	385
24.	Rammurti	32	60	9	Absent	Feminine	No	Present, B/L ++	Poorly oestrogenised.	Small size	65.8
25.	Pushpa	26	61	7	Absent	Feminine	No	Present, B/L +	Oestrogenised	Normal size	20.0

Patients of Primary infertility with primary amenorrhoea.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	Married period (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination		Serum Prolactin (in ng/ml
									Vagina	Uterus	
1.	Saroj	20	42	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.0
2.	Baby	28	49	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	19.5
3.	Satyawati	23	52	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	18.5
4.	Sushma	27	50	7	Absent	Feminine	No	Absent	Oestrogenised	Normal size	10.0
5.	Archna	20	61	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	8.5
6.	Aneeta	21	60	3	Absent	Feminine	No	Absent.	Poorly oestrogenised.	Small size	90
7.	Sangeeta	26	54	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22.0
8.	Rakhi	27	52	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	23.0
9.	Mamta	23	49	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	18.5
10.	Pranita	30	44	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.0
11.	Iti	21	51	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22.0
12.	Richa	26	58	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.0
13.	Purnima	32	52	10	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.0
14.	Ram Devi	25	54	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	8.5
15.	Kranti	24	55	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	12.0
16.	Leena	28	52	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.0
17.	Seema	22	61	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.5
18.	Rama	29	48	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	10.5

Patients of Primary infertility with polycystic ovarian disease.

1.	Sanju	26	48	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	35.5
2.	Muliya	22	46	2	Absent	Feminine	No	Absent	Oestrogenised	Small size	22
3.	Kushma	22	47	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	24.5

Patients of Primary infertility with Irregular menstruation.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	Married period (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination: Vagina	Vaginal examination: Uterus	Serum Prolactin (in ng/ml)
1.	Gonda	20	44	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	12
2.	Sangeeta	27	61	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	23
3.	Poonam	21	58	3	Absent	Feminine	No	Absent. Poorly oestrogenised.		Small size.	90
4.	Vinita	27	54	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20
5.	Padma	23	52	3	Absent	Feminine	No	Absent	Oestrogenised	Normal size	21
6.	Manikanta	28	57	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.5

Patients of Secondary infertility with irregular menses.

Sl. No.	Name	Age (yrs)	Wt. (kg)	LCB (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination: Vagina	Vaginal examination: Uterus	Serum Prolactin (in ng/ml)
1.	Suman	30	42	8	Absent	Feminine	No	Absent	Oestrogenised	Normal size	15.5
2.	Keranawati	31	49	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.5
3.	Deepa	30	54	5	Absent	Feminine	No	Absent	Oestrogenised	Small size	24
4.	Reeta	38	52	11	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22
5.	Mithlesh	33	57	10	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20

Patients of Primary infertility with Regular anovulatory cycles.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	Married period (yrs)	Headache & visual impairment	Appearance	Drug intake	Galactorrhoea	Vaginal examination: Vagina	Vaginal examination: Uterus	Serum Prolactin (in ng/ml)
1.	Phoolwati	20	48	2	Absent	Feminine	No	Absent	Oestrogenised	Normal size	20.5
2.	Veerwati	25	57	4	Absent	Feminine	No	Absent	Oestrogenised	Normal size	16.5
3.	Geeta	27	52	6	Absent	Feminine	No	Absent	Oestrogenised	Normal size	22

Patients of Secondary infertility with Regular anovulatory cycles.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	LCB (yrs)	Headache & visual impairment	Appear-ance	Drug intake	Galacto-rrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Rama	23	48		Present	Feminine	No	Absent	Hypo-oestrogenised.	Small size	362

Patients of Primary infertility with regular ovulatory cycles.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	Married period (yrs)	Headache & visual impairment	Appear-ance	Drug intake	Galacto-rrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Sheela	30	52		Absent	Feminine	No	Absent.	Normo-estrogenised.	Normal size	20.0
2.	Mamta	21	50		Absent	Feminine	No	Absent.	Normo-estrogenised.	Small size	5.5
3.	Brijchaya	27	46		Absent	Feminine	No	Absent	Hypo-estrogenised	Normal size	48
4.	Manju	26	48		Absent	Feminine	No	Absent	Normo-estrogenised.	Normal size	15.5

Patients of Secondary infertility with regular ovulatory cycles.

Sl. No.	Name	Age (yrs)	Wt. (kg.)	LCB (yrs)	Headache & visual impairment	Appear-ance	Drug intake	Galacto-rrhoea	Vaginal examination		Serum Prolactin (in ng/ml)
									Vagina	Uterus	
1.	Sanjana	30	58	5	Absent	Feminine	No	Absent	Hypo-oestrogenised.	Small uterus	60.5